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(57) Abstract

The present invention provides the nucleotide sequence of the M antigen gene of *H. capsulatum*, which is set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid having the nucleotide sequence complementary thereto, and nucleic acids having a nucleotide sequence which is substantially the same as the foregoing nucleotide sequences. The present invention also provides vectors and host expressions systems containing the foregoing nucleic acids, and isolated or recombinantly-produced antigens encoded by the foregoing nucleic acids. The present invention further provides antibodies generated against the foregoing antigens, and methods and kits for detecting a previous or current *Histoplasma capsulatum* infection in a subject, and for diagnosing histoplasmosis.

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NUCLEIC ACIDS OF THE M ANTIGEN GENE OF *HISTOPLASMA CAPSULATUM*, ANTIGENS, VACCINES AND ANTIBODIES, METHODS AND KITS FOR DETECTING HISTOPLASMOSIS

## 5 BACKGROUND OF THE INVENTION

## FIELD OF THE INVENTION

The present invention relates to reagents and methods for the detection of histoplasmosis. In particular, the present invention relates to nucleic acids (DNAs) relating to the M antigen gene of *Histoplasma capsulatum*; to vectors and host expression systems containing these nucleic acids; to nucleic acids (RNAs) which encode the M antigen of *H. capsulatum*; to isolated and recombinantly-produced antigens encoded by these nucleic acids; to antibodies produced against these antigens; to methods and kits for detecting histoplasmosis using these nucleic acids, antigens and antibodies; and to vaccines for the treatment or prevention of histoplasmosis.

## 20 BACKGROUND

Histoplasmosis is a systemic fungal disease resulting from the inhalation or, less frequently, the ingestion of spores of the fungus *Histoplasma capsulatum*, variety *capsulatum*, which is worldwide in distribution. The infection often causes acute pneumonia, or disseminated reticuloendothelial hyperplasia, or an influenza-like illness with joint effusion and erythema nodosum. Reactivated infection involves the lungs, meninges, heart, peritoneum and adrenals. Clinically inapparent or mild disease can result from limited, primary site infection of *H. capsulatum* in the lungs, but an often life-threatening, disseminated form of histoplasmosis can occur in immunodeficient patients, particularly the elderly, and those who have acquired immunodeficiency syndrome (AIDS). It is important to

properly identify *H. capsulatum* from other fungal species in order to determine the proper treatment for a fungal infection.

5 *H. capsulatum* is a dangerous, dimorphic, pathogenic fungus which, under different environmental conditions, may exist as either the yeast or mold phase. The organism exists as a multicellular mycelium at room temperature in rich soils, and in organic matter, in temperate environments worldwide, and proliferates as a  
10 unicellular yeast form at 37°C, and in infected host tissues. Only the yeast phase is known to survive within tissues, or within macrophages. The unicellular yeast form reproduces by budding on specialized media at 37°C. The mold form produces multicellular filamentous colonies  
15 that consist of cylindrical tubular structures called hyphae, and may contain microconidia and macroconidia which primarily grow under appropriate soil conditions, or on specialized fungal media, at 25°C. *H. capsulatum* occurs throughout the world, particularly in Brazil,  
20 Africa, India, Southeast Asia and the United States, but is most commonly found in soil from the fertile river valleys (Mississippi and Missouri river valleys) of the central United States.

*H. capsulatum* is associated with bird (particularly  
25 black bird and seagull) and bat excrement. (See, for example, Loyd et al., Histoplasma capsulatum. In Principles and Practice of Infectious Disease (3rd ed., Coordinating ed., Mandell et al., New York, (1990)); Wheat, "Diagnosis and Management of Histoplasmosis," *Eur.*  
30 *J. Clin. Microbiol. Infect. Dis.* 8:480 (1989).) The fungus infects the soil, and the resulting infected soil is often used as a habitat by birds and/or bats.

In addition to *H. capsulatum* var. *capsulatum*, two variants of Histoplasma exist: *H. capsulatum* var.  
35 *duboisii* (African histoplasmosis) and *H. capsulatum* var. *farcinosum* (epizootic lymphangitis of horses and

mules). (See, for example, Rippon, Histoplasmosis. In Medical Mycology The Pathogenic Fungi and the Pathogenic Actinomycetes (3rd ed., Saunders Company, Chapter 15 (1988)).) Many strains of *H. capsulatum* are currently  
5 deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, 20852. *H. capsulatum* strain CDC6623, deposited under accession number ATCC 26320, is discussed in Pine et al.,  
"Procedures for the Production and Separation of H and M  
10 Antigens in Histoplasmin, and Chemical and Serological Properties of the Isolated Products," *Mycopathologia* 61:131-141 (1977). The following other strains or variants of *H. capsulatum* are also deposited with the ATCC: *H. capsulatum* (attenuated Downs strain,  
15 filamentous phase, accession number ATCC 38904), *H. capsulatum* (attenuated Downs strain, yeast phase, accession number ATCC 38904), *H. capsulatum* (filamentous phase, accession number ATCC 11407), *H. capsulatum* (yeast phase, accession number ATCC 11407), *H. capsulatum* variant  
20 *duboisii* (filamentous phase, accession number ATCC 32281), *H. capsulatum* variant *duboisii* (yeast phase, accession number ATCC 32281), *H. capsulatum* variant *farciminosum* (filamentous phase, accession number ATCC 58332) and *H. capsulatum* variant *farciminasum* (yeast  
25 phase, accession number ATCC 58332).

The M antigen of *H. capsulatum* is a pluripotent glycoprotein having a molecular mass of 94 kDa, an isoelectric point of 4.7, oligosaccharide side chains, glycosidic epitopes which are N-linked to the peptide  
30 core, and protein epitopes, which have been shown to be unique to the *H. capsulatum* fungus. The peptide epitopes react with human antibodies, are not affected by N-deglycosylation, and trigger the proliferation of T cells. The M antigen is an immunodominant antigen of *H.*  
35 *capsulatum*, and elicits both humoral and cell-mediated immune responses. The glycopeptide bonds present in the glycoprotein are N linked. The M antigen of *H.*

*capsulatum* is considered to be the immunodominant antigen of *H. capsulatum* because antibodies generated against the M antigen are first to arise in infection, and are more commonly present during all phases of histoplasmosis.

5 Because the presence of this M antigen is indicative of histoplasmosis infection, the M antigen can serve as a marker for histoplasmosis infection. However, the biological identity of the M antigen has remained unknown. One report demonstrated that M protein was a  
10 catalase, based upon its ability to react with anti-catalase antibodies.

Currently, histoplasmosis is diagnosed by culture, or by the demonstration of a rise in complement-fixing antibody titers in serum. A definitive diagnosis of an  
15 *H. capsulatum* infection currently requires the isolation and propagation of the fungus, which is time-consuming and lacking in sensitivity, and which is dangerous for laboratory personnel, who must take extreme caution to prevent inhalation of the pathogenic fungus, so as not to  
20 become ill with a pulmonary infection. Further, only small quantities of antigens of *H. capsulatum* for use as biological reagents may be prepared in this manner.

Conventional laboratory identification methods used to isolate and identify *H. capsulatum* include the culture  
25 of a clinical specimen at room temperature on specialized fungal media. This procedure isolates the slower growing *H. capsulatum* colonies from possible contaminants, such as bacteria, and from faster growing saprobic fungi. This method, however, has several disadvantages. Because  
30 the growth of *H. capsulatum* to a visible colony normally takes from about two to four weeks, and sometimes as long as 12 weeks, this procedure is very slow. (See, for example, Rippon, Histoplasmosis. In Medical Mycology, The Pathogenic Fungi and the Pathogenic Actinomycetes,  
35 supra.; Koneman et al., Laboratory Identification of Molds, in Practical Laboratory Mycology, (3rd ed.

Williams & Wilkins (1985)); and McGinnis, Histoplasma capsulatum. In Laboratory Handbook of Medical Mycology (Academic Press (1986)).) Further, additional growth is required before the characteristic colony morphology and microscopic sporulation pattern with tuberculate macroconidia may be observed. In addition, approximately 10% of cultures produce only smooth-walled macroconidia, and some cultures fail to sporulate. Moreover, many species of fungi other than *H. capsulatum*, such as *Blastomyces dermatitidis*, *Chrysosporium* sp., and *Sepedonium* sp., produce similar colony and sporulation characteristics. Thus, additional testing is usually necessary to definitively identify the organism.

One method of converting the mycelial colony of *H. capsulatum* to the yeast phase is performed by subculturing the organism onto highly enriched cysteine-containing media, and incubating it at 35°-37°C. However, conversion to the yeast phase is often difficult, and may require several additional subcultures at three-day intervals.

Serologic evidence is the prime diagnostic indicator of histoplasmosis. Such evidence may be obtained with several serologic tests, such as the immunodiffusion test, which detects precipitants against the species-specific H and M antigens found in histoplasmin. (See, for example, Kaufman, "Laboratory Methods for the Diagnosis and Confirmation of Systemic Mycoses," *Clin. Infect. Dis.* 14:23-29 (1992), and Wheat, "Diagnosis and Management of Histoplasmosis," *supra*.)

Histoplasmin, an unpurified culture supernatant obtained from the mycelial phase of *H. capsulatum* grown in a chemically-defined medium containing *H. capsulatum* M antigens is currently used to probe both humoral and cell-mediated responses in patients with histoplasmosis. It is used for the serologic diagnosis of histoplasmosis, and as a skin test antigen to demonstrate delayed

hypersensitivity to infection in skin tests for histoplasmosis. The purification of histoplasmin is described by Bradley et al, "Purification, Composition, and Serological Characterization of Histoplasmin-H and M Antigen," *Infect. Immun.* 9:870-880 (1974). The preparation of H and M antigens of *H. capsulatum* free of heterologous antigens is described by Green et al., "Preparation of h and m Antigens of *Histoplasma capsulatum* Free of Heterologous Antigens," *Curr. Microbiol.* 12:209-216 (1985). (See also, Pine, "Histoplasma antigens: their Production, Purification and Uses," *Contrib. Microbiol. Immunol.* 3:138-168 (1977).) The preparation of antisera to the M antigen is described by Green et al., "H and M Antigens of *Histoplasma capsulatum*: Preparation of Antisera and Location of these Antigens in Yeast-Phase Cells," *Infect. Immun.* 14:826-831 (1976). General information concerning the serodiagnosis of fungal diseases is present in L. Kaufman et al., Serodiagnosis of Fungal Diseases, in Manual of Clinical Laboratory Immunology (3rd ed., American Society for Microbiology, Washington, D.C.(1988)).

Although the M antigen of *H. capsulatum* is useful in immunoassays for the diagnosis of histoplasmosis, purification of the M antigen from a batch culture is a laborious and low-yield process. The use of a recombinantly-produced M antigen of *H. capsulatum* in such immunoassays would significantly diminish the labor necessary to obtain M antigens which are pure enough to be useful in the immunoassays, and would result in high yields of the M antigen.

A need presently exists for biological reagents which can be produced and purified quickly and safely, and in large quantities, and which can be used in diagnostic assays to rapidly, easily and accurately detect a previous or current infection by *H. capsulatum*, and to diagnose histoplasmosis. A need also presently



exists for a method of rapidly, easily and accurately detecting a previous or current infection by *H. capsulatum*, and to diagnose histoplasmosis. Such biological reagents and methods would allow a clinician to improve the speed and accuracy of processing large numbers of clinical samples. Such reagents and methods would also aid the clinician in patient management, eliminate unnecessary tests, improve the speed, ease and accuracy of diagnosis and prognosis, help control histoplasmosis infection and reduce the use of unnecessary medications.

Accordingly, the present invention provides the DNA nucleotide sequence of the M antigen gene of *H. capsulatum*, and of related nucleotide sequences, which can be used to safely and rapidly produce, by recombinant DNA techniques, large quantities of the M antigen of *H. capsulatum* when inserted into a vector and placed into a suitable host for protein expression. The recombinantly-produced M antigens may be quickly and safely produced in large quantities in a pure, undegraded form. The present invention also provides the RNA nucleotide sequence which encodes the M antigen of *H. capsulatum*, and related nucleotide sequences. Nucleic acids, and fragments thereof, within the invention can also be used as nucleic acid probes in hybridization assays, or as primers in polymerase chain reaction assays, to detect *H. capsulatum* in clinical samples.

The present invention also provides the deduced amino acid sequence of the *H. capsulatum* M antigen. Isolated and recombinant M antigens encoded by nucleic acids within the present invention can be used as biological reagents in a wide variety of tests for histoplasmosis, such as skin tests, and immunoassays to detect a previous or current *H. capsulatum* infection in a tissue or fluid sample obtained from a human being or animal suspected of having, or having had,

histoplasmosis. For example, these antigens can be used as skin test antigens to ascertain the cell-mediated immune status of persons who have been exposed to *H. capsulatum*. The nucleic acids and antigens of the invention can also be used in a vaccine for the prevention or treatment of histoplasmosis.

The present invention also provides antibodies generated against the above antigens, which can be used in a wide variety of immunoassays to detect a current infection by *H. capsulatum*.

The present invention further provides methods for the detection of histoplasmosis, and related kits, using nucleic acids, antigens or antibodies within the invention.

The nucleic acids, vectors, hosts, isolated and recombinantly-produced antigens, antibodies, methods of detection and kits of the present invention permit the safe, direct, rapid, efficient, and accurate detection of a previous or current infection by *H. capsulatum* in a patient, and a positive diagnosis of histoplasmosis.

This patent application is believed to be the first report of the nucleotide sequence of the *H. capsulatum* M antigen gene, the nucleotide sequence which encodes the *H. capsulatum* M antigen, and of the amino acid sequence of the *H. capsulatum* M antigen.

#### DESCRIPTION OF THE RELATED ART

Zancopé-Oliveira et al., "Immunochemical Analysis of the H and M Glycoproteins from *Histoplasma Capsulatum*," *Clin. Diagn. Lab. Immunol.* Vol. 1, No. 5, 563-568 (1994), describes the use of different physicochemical methods to characterize the M and H antigens obtained from histoplasmin.

Fungal Pathogen *Histoplasma capsulatum*," *Microbiology* 140, 759-767 (1994), describes the cloning of the *H. capsulatum* yeast-phase-specific (*yps-3*) gene to clarify the mechanisms underlying pathogenesis and morphogenesis in the fungus *H. capsulatum*. The nucleotide sequence of the *yps-3* gene, and the predicted amino acid sequence of its product, are provided.

Deepe et al., "Immunobiological Activity of Recombinant H Antigen From *Histoplasma capsulatum*," *Infection and Immunity*, Vol. 63, No. 8, 3151-3157 (1995), describe the isolation and sequencing of the H antigen gene of *H. capsulatum*, and the recombinant production of the *H. capsulatum* H antigen in the bacterial expression vector pET 19b.

U.S. Patent No. 5,352,579 describes nucleic acid hybridization assay probes which are stated to be specific for *H. capsulatum* and no other fungi, and which have the nucleotide sequence 5'CGAAGTCGAGGCTTTCAGCATG3, or the nucleotide sequence complementary thereto. A probe having the above nucleotide sequence is stated to hybridize to the 18S rRNA of *H. capsulatum* corresponding to bases 172-193 of *Sacchomyces cerevisiae*. This patent also describes the use of helper probes having the sequence 5'TATTAGCTCTAGAATTACCACGGGTATCCAAGTAGTAAGG3, or the sequence 5'CCCCGAAGGGCATTGGTTTTTTTATCTAATAAATACACCCC3'.

None of the above documents teaches or suggests the DNA nucleotide sequence of the *H. capsulatum* M antigen gene, the RNA nucleotide sequence which encodes the *H. capsulatum* M antigen, the amino acid sequence of the *H. capsulatum* M antigen, or the production of the *H. capsulatum* M antigen using recombinant DNA techniques.

#### SUMMARY OF THE INVENTION

The present invention provides the nucleotide sequence of the M antigen gene (DNA) of the *Histoplasma*

*capsulatum* species of fungus, which is set forth in the Sequence Listing as SEQ ID NO:1.

The present invention also provides a nucleic acid specific to *Histoplasma capsulatum* comprising a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or a fragment of a nucleic acid having a nucleotide sequence which is substantially the same as a nucleic acid which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1. The isolated nucleic acid of this invention does not contain the nucleotide sequence 5'CGAAGTCGAGGCTTTCAGCATG3, the nucleotide sequence complementary thereto, the nucleotide sequence 5'TATTAGCTCTAGAATTACCACGGGTATCCAAGTAGTAAGG3, the nucleotide sequence complementary thereto, the nucleotide sequence 5'CCCCGAAGGGCATTGGTTTTTTATCTAATAAATACACCCC3, or the nucleotide sequence complementary thereto. Further, the isolated nucleic acid is not a nucleic acid consisting

essentially of between 10 and 100 nucleotides which is able to form a hybrid at 60°C with a nucleotide polymer having a nucleotide base sequence of

5 CGAAGTCGAGGCTTTCAGCATG3 , 5 CATGCTGAAAGCCTCGACTTCG3 ,  
5 CAUGCUGAAAGCCUCGACUUCG3 or 5 CGAAGUCGAGGCUUUCAGCAUG3 .

The present invention further provides the amino acid sequence of the isolated or recombinantly-produced M antigen of the *Histoplasma capsulatum* species of fungus, which is set forth in the Sequence Listing as SEQ ID  
10 NO:2. The antigen is encoded by a nucleic acid (RNA) having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

The present invention also provides an isolated or  
15 recombinantly-produced antigen specific to *Histoplasma capsulatum* comprising a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a fragment of a polypeptide  
20 encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a  
25 nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary  
30 to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

The invention further provides monoclonal or polyclonal antibodies generated against one of the isolated or recombinantly-produced antigens described  
35 above.

The present invention also provides a vector comprising a nucleic acid specific to *Histoplasma capsulatum*, wherein the nucleic acid has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, has a nucleotide sequence which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, is a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, and wherein the vector is suitable for expressing the nucleic acid.

The present invention still further provides a host for expressing an antigen which is specific to *Histoplasma capsulatum* comprising a vector containing a nucleic acid, wherein the vector is suitable for expressing the nucleic acid, and wherein the nucleic acid is as described above for the vector of the present invention.

The present invention also provides a vaccine for the treatment or prevention of histoplasmosis comprising: (a) a nucleic acid, or an isolated or recombinantly-produced antigen, which is specific to *Histoplasma capsulatum*; and (b) a pharmaceutically-acceptable carrier for the nucleic acid or antigen, wherein the nucleic acid in a nucleic acid as described above, and wherein the antigen is an antigen as described above.

The present invention further provides a method for detecting a previous or current *Histoplasma capsulatum* infection in a subject, comprising: (a) contacting a fluid or tissue sample from the subject which contains antibodies with an isolated or recombinantly-produced antigen which is specific to *Histoplasma capsulatum*; and (b) detecting the presence of binding between the

antibodies and the antigen, the presence of binding indicating the presence of a previous or current *Histoplasma capsulatum* infection in a subject, wherein the antigen is one of the antigens described above.

5       The present invention further provides a method for detecting a past exposure to the fungus *Histoplasma capsulatum* comprising: (a) injecting intradermally in the skin of a subject a liquid containing an isolated or recombinantly-produced antigen which is specific to  
10 *Histoplasma capsulatum*; and (b) observing the skin of the subject at the injection site at one or more predetermined times after injection for the presence of swelling of the skin, the presence of swelling of the skin indicating a past exposure by the subject to the  
15 fungus *Histoplasma capsulatum*, wherein the antigen is one of the antigens described above.

      The present invention still further provides a kit for detecting a previous or current *Histoplasma capsulatum* infection in a sample comprising: (a) a  
20 nucleic acid, an isolated or recombinantly-produced antigen, or an antibody described above; and (b) instructions describing the use of the nucleic acid, antigen or antibody in the detection of a previous or current *Histoplasma capsulatum* infection.

25       The present invention also provides a method for detecting a current *H. capsulatum* infection in a subject suspected of having an *H. capsulatum* infection comprising: (a) contacting a fluid or tissue sample from the subject which contains antigens with antibodies  
30 generated against an antigen which contains an epitope which is unique to *H. capsulatum*; and (b) detecting the presence of binding between the antigens and the antibodies, the presence of binding indicating the presence of a current *H. capsulatum* infection in the  
35 subject, wherein the antigen is one of the antigens described above.

### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention, and to the  
5 Example and Sequence Listing included therein.

#### Definitions

The phrases "specific to" and "unique to" the fungus *H. capsulatum* as used herein in relation to an antigen means that the antigen (an antigenic polypeptide or  
10 polypeptide fragment) contains at least one epitope which is not common to other related fungi or other microorganisms (i.e., it is unique to the fungus *H. capsulatum*), and binds with a higher affinity to antibodies generated against antigens of the fungus *H.*  
15 *capsulatum* than with antibodies generated against other related fungi or microorganisms. Thus, such an antigen can be distinguished from other antigens by such higher binding affinity. The phrases "specific to" and "unique to" the fungus *H. capsulatum* as used herein in relation  
20 to a nucleic acid or nucleic acid fragment means a nucleic acid or nucleic acid fragment which is not common to other related fungi or other microorganisms (i.e., it is only present in the fungus *H. capsulatum*).

The phrase "fully complementary" as used herein  
25 refers to a nucleic acid which is both the same length as, and exactly complementary in base pairing to, a given nucleic acid.

The phrase "fluid or tissue sample" as used herein means any sample of fluid, or of solubilized or  
30 nonsolubilized tissue, obtained from a subject, or solubilized or nonsolubilized cultured cells, which



contains components, such as nucleic acids, antibodies or antigens, or fragments thereof, which may be employed in one of the tests described herein to detect a previous or current infection by, or exposure to, the fungus *H. capsulatum*, or to make a positive diagnosis of histoplasmosis. Such fluid or tissue samples include blood, serum, plasma, sputum, urine, mucus, saliva, gastric juice, lymph, feces, or other bodily fluids, and tissues from the lungs, spleen, liver, skin or other organs. The tissue or fluid samples can also be supernatant from incubated tissue samples or cultured cells.

The term "fragment" as used herein in relation to a polypeptide means a subsequence of the polypeptide which is of a sufficient size and conformation to remain immunogenic (i.e., to have at least one epitope) and/or to produce swelling of the skin of a subject in a skin test for histoplasmosis. The term "fragment" as used herein in relation to a nucleic acid means a subsequence of the nucleic acid which is of a sufficient size and confirmation to properly function as a hybridization probe, as a primer in a polymerase chain reaction, to code for a polypeptide or polypeptide fragment, or in another manner characteristic of nucleic acids.

The term "hybridization" as used herein refers to the formation of a duplex structure by two single-stranded nucleic acids due to fully (100%) or less than fully (less than 100%) complementary base pairing. Hybridization can occur between fully complementary nucleic acid strands, or between less than fully complementary nucleic acid strands which contain regions of mismatch due to one or more nucleotide substitutions, deletions or additions.

The terms "immunogenic" and "antigenic" as used herein mean that a polypeptide, or a fragment thereof, elicits a protective immune response, for example, the production of antibodies against the polypeptide, or  
5 fragment thereof, in a subject to which it is administered. The polypeptide or polypeptide fragment will have at least one epitope present therein.

The term "isolated" means that the nucleic acids, nucleic acid fragments, polypeptides, polypeptide  
10 fragments or antibodies are of sufficient purity so that they may be employed, and will function properly, in a clinical, diagnostic, experimental or other procedure, such as an immunoassay, a hybridization assay, an amplification reaction, or a skin test for  
15 histoplasmosis. Many procedures are known by those of ordinary skill in the art for purifying nucleic acids, nucleic acid fragments, polypeptides, polypeptide fragments and antibodies from other proteins, contaminants, and materials with which they may normally  
20 be associated prior to their use in various procedures. For example, the M antigen of *H. capsulatum* obtained from histoplasmin may be purified by standard chromatography procedures, such as cation-exchange chromatography or anion-exchange chromatography, to remove other antigens  
25 (c and h antigens, etc.) and proteins, and other components, of histoplasmin therefrom. Recombinantly-produced *H. capsulatum* M antigen may be purified by bound nickel-ion exchange chromatography, or by a combination of Fast Protein Liquid Chromatography (FPLC) using size  
30 exclusion chromatography and anion and/or cation exchange chromatography.

Abbreviations for "nucleotides" used herein follow the nomenclature described by the Nomenclature Committee for the International Union of Biochemistry,

"Nomenclature for Incompletely Specified Bases in Nucleic Acid Sequences," *Eur. J. Biochem.* 150:1-5 (1985), in which "A" represents adenine residues, "C" represents cytosine residues, "T" represents thymine residues, "G" represents guanine residues, "I" represents deoxyinosine residues, "M" represents adenine or cytosine residues, "R" represents adenine or guanine residues and "Y" represents cytosine or thymine residues.

The terms "nucleic acid" and "oligonucleotide" include polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), and to any other type of polynucleotide which is an N glycoside of a purine or pyrimidine base, or modified purine or pyrimidine base. The terms "nucleic acid" and "oligonucleotide" are used interchangeably herein. These terms refer only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, as well as double- and single-stranded RNA. Nucleic acids and oligonucleotides can be prepared by any of several well-known methods. For example, they may be prepared by cloning and restriction of desired sequences, or by direct chemical synthesis by the phosphotriester methods described by Narang et al., *Meth. Enzymol.* 68:90-99 (1979) and Brown et al., *Meth. Enzymol.* 68:109-151 (1979); by the diethylphosphoramidite method described by Beaucage et al., *Tetrahedron Lett.* 22:1859-1862 (1981); or by the solid support method described in U.S. Patent No. 4,458,066. A review of nucleic acid syntheses methods is provided in Goodchild, *Bioconjugate Chemistry* 1(3):165-187 (1990).

The term "polypeptide" as used herein means a sequence of four or more amino acids which is immunogenic and/or produces swelling of a subject's skin in a skin test for histoplasmosis, for example the M antigen protein of *H. capsulatum*. The sequence of four or more

amino acids can be modified, for example, by chemical, enzymatic or other treatment which does not diminish the immunogenic activity of the polypeptide to any substantial extent.

- 5           The phrase "recombinant DNA techniques" as used herein means well-known techniques which permit the isolation and propagation of individual genes, such as the M antigen gene of *H. capsulatum*, and the efficient expression of their products, such as the M antigen of *H.*
- 10 *capsulatum*, by plasmid or other expression vectors in various bacterial, yeast or mammalian host expression systems. General information concerning recombinant DNA techniques is present, for example, in Rodriguez et al., Recombinant DNA Techniques: An Introduction (The
- 15 Benjamin/Cummings Publishing Company, Inc., Menlo Park, California, 1983), in Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982), and in Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing
- 20 Associates and Wiley-Interscience, (John Wiley and Sons, New York (1987; updated quarterly)). The phrase "recombinantly-produced" as used herein means produced by recombinant DNA techniques.

- 25           The term "substantially the same as" in relation to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or to the nucleotide sequence fully complementary thereto, refers to a nucleic acid having a nucleotide sequence which is similar to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or to the nucleotide
- 30 sequence which is fully complementary thereto, and which retains the functions of such nucleic acid, but which differs from such nucleic acid by the substitution, deletion and/or addition of one or more nucleotides, and/or by the

incorporation of some other advantageous feature into the nucleic acid, such as a radio label or other label (biotin, etc.) for nucleic acid detection or immobilization. For example, the essential structure and function of a polypeptide or polypeptide fragment  
5 encoded by a nucleic acid which is substantially the same as the above nucleic acids should be the same as the structure and function of a polypeptide or polypeptide fragment encoded by the above nucleic acids. Generally, these nucleic acids will have a nucleotide sequence which has less than about 10% divergence from the nucleotide  
10 sequence set forth in the Sequence Listing as SEQ ID NO:1, or from the nucleotide sequence which is fully complementary thereto. Preferably, the nucleic acids will have about 90%, or more preferably about 95%, or even more preferably about 99% homology with the nucleotide sequence set forth in the Sequence Listing as SEQ ID  
15 NO:1, or with the nucleotide sequence fully complementary thereto.

Due to the degeneracy in the genetic code, a sequence of three nucleotides (a codon) codes for each of the twenty natural amino acids. However, because there are twenty amino acids and sixty-four possible codons, most amino acids are specified by more  
20 than one codon. Thus, the nucleotide sequence of the *H. capsulatum* M antigen gene may be varied from the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, and the nucleotide sequence which encodes the *H. capsulatum* M antigen may be varied from the nucleotide sequence which is fully complementary to the nucleotide  
25 sequence set forth in the Sequence Listing as SEQ ID NO:1. Thus, nucleic acids within the present invention are not limited to nucleic acids having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or having a nucleotide sequence fully complementary thereto.

30 The nucleic acids of the present invention will have the ability of the nucleic acid whose nucleotide sequence is set forth in the Sequence Listing as SEQ ID NO:1, or

whose nucleotide sequence is fully complementary thereto, to encode the *H. capsulatum* antigen gene, or M antigen product of this gene, with the M antigen being specific to *H. capsulatum* and being antigenic (being able to stimulate the production of antibodies against the antigen). Alternatively, the nucleic acids of the present invention will have the ability to function as hybridization probes, or as primers in amplification reactions, for the detection of *H. capsulatum*.

Modifications at the 5'- end of a nucleic acid can include, for example, the addition of an isotope, such as <sup>32</sup>P, or a chemical, such as digoxigenin, for detection when using a commercial kit, such as the Boehringer-Mannheim Dig/Genius detection system. In addition, restriction enzyme sites and/or cloning sites can be

added to the 5'- end of a nucleic acid (from about 6 to more than about 12 nucleotides) for the direct cloning of the amplified product.

The phrases "target region" and "target nucleic acid" refer to a region of a nucleic acid which is to be amplified, detected, or otherwise analyzed. The sequence to which a primer hybridizes is referred to as a "target sequence."

#### Nucleic Acids

In one aspect, the present invention provides nucleic acids which are specific to the fungus *H. capsulatum*.

Examples of the nucleic acids of the present invention include a DNA having the nucleotide sequences set forth in the Sequence Listing as SEQ ID NO:1, an RNA having a nucleotide sequence which is fully complementary

to the DNA nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, and fragments of the foregoing nucleic acids.

#### Modified Nucleic Acids

5 Nucleic acids within the present invention also include nucleic acids which are substantially the same as the nucleic acids having the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or the  
10 nucleotide sequence which is fully complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

Modifications to a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1, or to a nucleic acid having a nucleotide sequence  
15 which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, such as one or more nucleotide substitutions, additions, and/or deletions, or the addition of some beneficial component  
to the nucleic acid, such as a radiolabel or non-  
20 radiolabel for nucleic acid detection or immobilization, can be made so long as the nucleic acids do not lose their ability to function in one of the manners described herein. Such modified nucleic acids are within the scope of the present invention if they have the ability to  
25 function to encode the *H. capsulatum* M antigen gene, to encode an antigenic polypeptide which is specific to *H. capsulatum*, to function as a nucleic acid probe in a hybridization assay for the detection of *H. capsulatum*, to function as a primer in a polymerase chain reaction  
30 used to detect *H. capsulatum*, or to function in some other manner which is characteristic of nucleic acids.

Computer programs are readily available to the skilled artisan which can be used to compare modified nucleotide sequences to previously published nucleotide  
35 sequences of *H. capsulatum* to select appropriate sequences for use. A computerized comparison of modified

sequences with known sequences catalogued in GENBANK, a computer-  
ized database, may be made using the commercially-available com-  
puter programs DNASIS (Hitachi Engineering, Inc.), Word Search or  
FASTA of the Genetics Computer Group (Madison, WI), which search  
5 the catalogued nucleotide sequences for similarities to the nucleic  
acid in question.

#### Nucleic Acid-Based Assay Techniques

The nucleic acids of the present invention can be used to  
detect a current *H. capsulatum* infection in a sample by any of a  
10 number of well-known nucleic acid-based detection techniques, such  
as hybridization techniques, polymerase chain reaction (PCR),  
reverse transcription polymerase chain reaction (RT-PCR), ligase  
chain reaction (LCR), nucleic acid sequencing techniques, electro-  
phoretic and non-electrophoretic identification of nucleic acids,  
15 and the like. Alternatively, these nucleic acids can also be used  
in vectors to safely produce large quantities of the *H. capsulatum*  
M antigen in suitable host cells for use in the immunodiagnostic  
techniques and skin tests for histoplasmosis described herein.  
Thus, the nucleic acids of the present invention, which can vary in  
20 length, can be used as probes in nucleic hybridization assays for  
the detection of *H. capsulatum*, or as primers in polymerase chain  
reactions for the detection of *H. capsulatum*. It is also contem-  
plated that the nucleic acids of the present invention can be  
labeled or tagged for use in radioactive, chemiluminescence,  
25 fluorescent, or other detection systems.

*H. capsulatum* infection in a tissue or fluid sample suspected  
of containing *H. capsulatum* infection may be detected by detecting  
nucleic acids of *H. capsulatum*. Based upon the nucleotide sequence  
set forth in SEQ ID NO:1, one can design reagents by known methods  
30 to detect the presence of *H. capsulatum* in a sample. For example,  
DNA or RNA obtained from a sample suspected of containing



*H. capsulatum* can be sequenced by known methods, and the sequence compared to the nucleotide sequence set forth in SEQ ID NO:1. If the sequence of DNA or RNA obtained from the sample has greater than about 10% divergence from the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or from a nucleotide sequence complementary thereto, then the sample does not contain *H. capsulatum*. Otherwise (if there is about 90% or more sequence similarity between DNA or RNA obtained from the sample and the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or the nucleotide sequence complementary thereto), a positive diagnosis of current infection in the sample by the fungus *H. capsulatum* can be made. The above-described computer programs may be used to make the nucleotide sequence comparisons.

Amplification reactions can also be used for detecting *H. capsulatum* infection in a sample. DNA obtained from the sample can be amplified using nucleic acid primers specific to *H. capsulatum*, and detecting the presence of a nucleic acid which is unique to *H. capsulatum*. The presence of a nucleic acid which is unique to *H. capsulatum* indicates the presence of *H. capsulatum* in the sample. The detection of a nucleic acid which is unique to *H. capsulatum* can be by the detection of amplification product when *H. capsulatum*-specific primers are used. The detection of a nucleic acid unique to *H. capsulatum* can be performed by direct hybridization utilizing a *H. capsulatum*-specific oligonucleotide probe, or by a restriction fragment length polymorphism. The primers (and probes) can, for example, be derived from the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, or the sequence complementary thereto. Particularly useful regions of the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1 for such purpose are (1) the DNA at the amino terminus encoding amino acids

1-24 of the M antigen, or the sequence complementary thereto; and (2) the DNA at the carboxy terminus encoding amino acids 601-707 of the M antigen, or the sequence complementary thereto. Standard criteria for the selection of sequences for primer development are applicable. The crucial requirement is that the primers be such that an amplification protocol using them can distinguish *H. capsulatum* nucleic acids from the nucleic acids of other fungi, and other microorganisms. While non-specific amplification may also occur, the skilled artisan can distinguish non-specific amplification from the amplification of nucleic acids of *H. capsulatum*, for example, by following amplification with the use of a specific probe derived from the nucleotide sequence set

forth in the Sequence Listing as SEQ ID NO:1, or to a sequence fully complementary thereto.

For such uses, the nucleic acids are typically between about 10 and about 100 nucleotides in length, preferably between about 12 and about 30 nucleotides in length, and most preferably between about 15 and about 25 nucleotides in length. There is no standard length for optimal hybridization or polymerase chain reaction amplification. An optimal length for a particular primer application may be readily determined in the manner described in H. Erlich, PCR Technology, Principles and Application for DNA Amplification, (1989). Several computer software programs are available to facilitate primer design, for example, Lowe, "Computer Program for Selection of Oligonucleotide Primers for Polymerase Chain Reactions," *Nucl. Acids. Res.* 18:1757-1761 (1991) and RT-PCR, Methods and Applications Book 1, (Clontech Laboratories, Inc. (1991)).

In particular, an isolated nucleic acid that selectively hybridizes with (or selectively amplifies) the nucleic acid set forth in SEQ ID NO:1, or the nucleic acid fully complementary thereto, under stringent

conditions, and comprises at least 10 nucleotides complementary to the sequence set forth in SEQ ID NO:1, or the nucleic acid fully complementary thereto, is provided. The hybridizing nucleic acid should have at least about 97% (and preferably about 98% or 99%) complementarity with the segment of the nucleic acid of SEQ ID NO:1, or the nucleic acid fully complementary thereto, to which it hybridizes. As used herein to describe nucleic acids, the term "selectively hybridizes" means that a nucleic acid hybridizes with a particular nucleotide sequence, and not with others, and excludes the occasional randomly hybridizing nucleic acids. The hybridizing nucleic acids can be used, for example, as probes or primers for detecting an isolate of *H. capsulatum* that has the nucleic acid to which the primer or probe hybridizes. Thus, these nucleic acids can be the coding sequence for the *H. capsulatum* M antigen protein, or for fragments thereof, that can be utilized to produce an antigenic protein or protein fragment.

If used as primers, the invention provides compositions including at least two nucleic acids which hybridize with different regions of the target *H. capsulatum* sequence so as to amplify a desired region of the target *H. capsulatum* sequence. Depending on the length of the probe or primer, the target region can range from about 97% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for the purpose of diagnosing the presence of *H. capsulatum* infection, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (e.g., *H. capsulatum* DNA from a sample) is at least enough to distinguish hybridization with a nucleic acid from related fungi.

In general, the nucleic acids of the present invention may be prepared and tested for the ability to selectively hybridize with a target nucleic acid in the

manner described herein, or by modifications thereof, using readily-available starting materials, reagents and equipment.

5       The polymerase chain reaction (for amplifying DNA) and the reverse transcription polymerase chain reaction (for amplifying cDNA generated from RNA) are rapid methods for increasing the copy number of, and sensitively detecting, specific nucleic acid sequences. These methods may be used for the rapid detection of *H.*  
10   *capsulatum* from clinical samples.

      The nucleic acids present in a sample which are being amplified may be a single- or double-stranded DNA or RNA. If the starting material is RNA, reverse transcriptase is used to prepare a first strand cDNA  
15   prior to conventional polymerase chain reaction.

      General information concerning polymerase chain reaction, and the amplification of specific sequences of nucleic acids, is present in U.S. Patent No. 4,683,195; U.S. Patent No. 4,683,202; U.S. Patent No. 4,965,188; U.S. Patent No. 5,578,467; U.S. Patent No. 5,545,522; U.S. Patent No. 5,624,833; Ausubel et al., Current  
20   Protocols in Molecular Biology, supra.; Rotbart, "Enzymatic RNA Amplification of the Enteroviruses," *J. Clin. Microbiol.* 28:438-442 (1990); Kawasaki, "Amplification of RNA," 21-27, in M. Innis et al., PCR  
25   Protocols (Academic Press, New York (1990)); and Rossolini et al., "Use of Deoxyinosine-Containing Primers vs. Degenerate Primers for Polymerase Chain Reaction Based on Ambiguous Sequence Information," *Mol. Cell*  
30   *Probes* 8:91-98 (1994). The amplification of cDNA generated from RNA using a reverse transcription/polymerase chain reaction is described in U.S. Patent No. 5,310,652 and U.S. Patent No. 5,322,770. Commercial vendors, such as Perkin Elmer (Norwalk, Connecticut),  
35   market polymerase chain reaction reagents and equipment and publish suitable protocols.

In each cycle of an amplification reaction, a double-stranded target nucleic acid sequence present in a sample is denatured and, due to the presence of a large molar excess of the primers, primers are annealed to each strand of the denatured target sequence. The primers, oriented with their 3' ends pointing towards each other, hybridize to opposite strands of the target sequence and, due to the action of DNA polymerase, prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The two primers anneal to opposite ends of the target nucleic acid sequence, and in orientations such that the extension product of each primer is a complementary copy of the target nucleic acid sequence and, when separated from its complement, can hybridize to the other primer. The end product is then denatured again for another cycle. After this three-step cycle has been repeated between about 25 and 40 times, amplification of a nucleic acid segment by more than one million-fold can be achieved. Each cycle, if 100% efficient, would result in a doubling of the number of target sequences present, thereby leading to exponential increases in the concentration of desired nucleic acid sequences. Better amplification is generally obtained when both primers are approximately the same length.

Denaturation of nucleic acid strands usually takes place at about 94°C. The normal annealing (55 to 60°C) and extension (65 to 72°C) temperatures generally used for *in vitro* amplification by polymerase chain reaction may be used. Examples of suitable reaction times are from about 30 seconds to about 1 minute denaturing; from about 30 seconds to about 1 minute of annealing; and from about 30 seconds to about 2 minutes of extension. One of ordinary skill in the art can, of course, easily determine optimum reaction times and conditions using conventional techniques.

Suitable assay formats for detecting amplification products or hybrids formed between probes and target nucleic acid sequences in a sample are described, for example, in Ausubel et al., Current Protocols in Molecular Biology, supra., and in Sambrook et al., Molecular Cloning-A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1985). Examples of these assay formats include the dot-blot and reverse dot-blot assay formats. In a dot-blot format, amplified target DNA is immobilized on a solid support, such as a nylon membrane. The membrane-target complex is incubated with labeled probe under suitable hybridization conditions, unhybridized probe is removed by washing under suitable stringent conditions, and the membrane is monitored for the presence of bound probe. In a "reverse" dot-blot format, in which the amplified target DNA is labeled and the probes are immobilized on a solid support (e.g., nylon membrane). The target DNA is typically labeled during amplification by the incorporation of labeled primers therein. One or both of the primers can be labeled. The membrane-probe complex is incubated with the labeled amplified target DNA under suitable hybridization conditions, unhybridized target DNA is removed by washing under suitably stringent conditions, and the filter is then monitored for the presence of bound target DNA.

"Stringent conditions" refers to the hybridization conditions used in a hybridization protocol, for example, DNA/DNA hybridization, or in the primer/template hybridization in a PCR reaction. In general, these conditions should be a combination of temperature and salt concentration for washing chosen so that the denaturation temperature is approximately 5-20°C below the calculated  $T_m$  (melting/denaturation temperature) of the hybrid under study. The temperature and salt conditions are readily determined empirically in

preliminary experiments in which samples of reference DNA are hybridized to the primer nucleic acid of interest, and then amplified under conditions of different stringencies. The stringency conditions are easily  
5 tested, and the parameters altered will be apparent to one skilled in the art. For example,  $MgCl_2$  concentrations used in the reaction buffer can be altered to increase the specificity with which the primer binds to the  
10 template, but the concentration range of this compound used in hybridization reactions is narrow and, therefore, the proper stringency level is easily determined. For example, hybridizations with oligonucleotide probes 18 nucleotides in length can be done at 5-10°C below the estimated  $T_m$  in 6X SSPE, then washed at the same  
15 temperature in 2X SSPE. (See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, supra.) The  $T_m$  of such an oligonucleotide can be estimated by allowing 2°C for each A or T nucleotide, and 4°C for each G or C. An 18 nucleotide probe of 50% G+C would, therefore, have an  
20 approximate  $T_m$  of 54°C. Likewise, the starting salt concentration of an 18 nucleotide primer or probe would be about 100-200mM. Thus, stringent conditions for such an 18 nucleotide primer or probe would be a  $T_m$  of about 54°C, and a starting salt concentration of about 150 mM,  
25 and modified accordingly by preliminary experiments.  $T_m$  values can also be calculated for a variety of conditions utilizing commercially available computer software (e.g., OLIGO®).

Conventional techniques of molecular biology and  
30 nucleic acid chemistry which may be employed in the preparative and testing processes of the present invention are fully explained in the literature. See, for example, Ausubel et al., Current Protocols in Molecular Biology, supra. Sambrook et al., Molecular Cloning-A Laboratory Manual, supra.; Watson et al.,  
35 Molecular Biology of the Gene (Fourth Edition, The

Benjamin/Cummings Publishing Company, Inc. 1987); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); and Nucleic Acid Hybridization (B. D. Hames and S. J. Higgins. eds., 1984).

5    **Vectors and Hosts**

          The present invention also provides a vector comprising a nucleic acid having the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, having a nucleotide sequence which is substantially the same as  
10   the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1, a nucleic acid complementary to, or capable of hybridizing with, either of the foregoing nucleic acids, or a fragment of any of the foregoing nucleic acids. The vectors of the invention can be  
15   placed into a host (e.g., cell line or transgenic animal) that can express the polypeptides and polypeptide fragments of the present invention.

          The *H. capsulatum* M antigen gene (and other nucleic acids within the invention) can be cloned into suitable  
20   expression vectors by linking the gene to a suitable promoter in a replicable vector, and expressed in various bacterial, yeast or mammalian host expression systems, as is described in the Example, to safely produce large quantities of the *H. capsulatum* M antigen by propagating  
25   the vector in the host under conditions conducive to protein expression. Using conventional techniques, a DNA sequence containing the *H. capsulatum* M antigen gene can be cloned from *H. capsulatum* genomic DNA. The DNA can be converted to double-stranded DNA using cloning techniques  
30   well known in the art, including PCR techniques. Linkers or tails may be placed on the ends of the double-stranded DNA to provide convenient restriction sites. After restriction digestion, the DNA may be introduced to any site in a vector, such as a plasmid vector, which has  
35   been restricted with a restriction enzyme which generates



compatible ends. Following ligation, by means of standard techniques, the DNA can then be introduced into a suitable host system, where it can be expressed to produce the desired *H. capsulatum* M antigen protein.

5        If desired, the coding sequence for the *H. capsulatum* M antigen gene can be subjected to site-specific mutagenesis, in the manner discussed by Maniatis et al., Molecular Cloning: A Laboratory Manual, supra., to alter selected base pairs. Oligonucleotides  
10       containing a mutation to be introduced to the cloned gene can be synthesized by well-known DNA synthetic techniques, preferably by phosphorasmidite chemistry, and preferably as implemented on an automated synthesizer, such as the synthesizer commercialized by Applied  
15       Biosystems.

         There are numerous *E. coli* expression vectors known to those of ordinary skill in the art which are useful for the expression of the polypeptides and polypeptide fragments of the invention. Other microbial hosts  
20       suitable for such use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia* and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors which contain expression control sequences  
25       compatible with the host cell, such as an origin of replication. In addition, any number of a variety of well-known compatible promoters will be present, such as a lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter  
30       system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences for initiating and completing transcription and translation. If necessary, an amino terminal methionine can be provided  
35       by the insertion of a Met codon 5' in-frame with the polypeptide or polypeptide fragment. Also, the carboxyl-

terminal extension of the antigen can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression systems can be used for the recombinant production of the polypeptide or polypeptide fragment. There are several advantages to the use of yeast expression systems for this purpose. First, evidence exists that proteins produced in a yeast secretion system generally exhibit correct disulfide pairing. Second, post-translational glycosylation is generally efficiently carried out by yeast secretory systems. The *Saccharomyces cerevisiae* pre-pro-alpha-factor leader region (encoded by the *MF $\alpha$ -1* gene) is routinely used to direct protein secretion from yeast. (See, for example, Brake et al., " $\alpha$ -Factor-Directed Synthesis and Secretion of Mature Foreign Proteins in *Saccharomyces cerevisiae*," *Proc. Nat. Acad. Sci.* 81:4642-4646 (1984)). The leader region of pre-pro-alpha-factor contains a signal peptide and a pro-segment which includes a recognition sequence for a yeast protease encoded by the *KEX2* gene. This enzyme cleaves the precursor protein on the carboxyl side of a Lys-Arg dipeptide cleavage-signal sequence. The antigen coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This construct is then put under the control of a strong transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The antigen coding sequence is followed by a translation termination codon, which is followed by transcription termination signals. Alternatively, the antigen coding sequences can be fused to a second protein coding sequence, such as S<sub>j</sub>26 or  $\beta$ -galactosidase, used to facilitate purification of the fusion protein by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion protein is applicable to constructs used for expression in yeast. Efficient post translational glycosylation and expression

of recombinant proteins can also be achieved in Baculovirus systems.

Mammalian cells permit the expression of proteins in an environment which favors important post-translational modifications, such as folding and cysteine pairing, the addition of complex carbohydrate structures, and the secretion of active protein. Vectors useful for the expression of antigen in mammalian cells are characterized by insertion of the antigen coding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or methotrexate resistance for use as selectable markers. The antigen coding sequence can be introduced, for example, into a Chinese hamster ovary cell line using a methotrexate resistance-encoding vector. The presence of the vector DNA in transformed cells can be confirmed by Northern blot analysis, and the production of an opposite strand RNA corresponding to the antigen coding sequence can be confirmed by Southern blot analysis. A number of other suitable host cell lines capable of secreting intact human proteins have been developed, and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, and the like. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus and Bovine Papilloma Virus. The vectors containing the nucleic acid segments of interest can be transferred into the host cells by well-known methods, which vary depending upon the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium

phosphate treatment or electroporation may be used for other cellular hosts.

Alternative vectors for the expression of antigen in mammalian cells, such as those which are similar to the  
5 vectors developed for the expression of human gamma-interferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease Naxin1, and eosinophil major basic protein, can also be employed. Further, the vector can include CMV promoter sequences  
10 and a polyadenylation signal available for expression of inserted nucleic acid in mammalian cells, such as COS7.

The nucleic acid sequences can be expressed in hosts after the sequences have been operably linked, i.e., positioned, to ensure the functioning of an expression  
15 control sequence. These expression vectors are typically replicable in the host organisms either as episomes, or as an integral part of the host chromosomal DNA. Commonly, expression vectors can contain selection markers, e.g., tetracycline resistance or hygromycin  
20 resistance, to permit detection and/or selection of those cells transformed with the desired nucleic acid sequences. (See, for example, U.S. Patent 4,704,362).

Nucleic acids encoding a variant polypeptide may include sequences which facilitate transcription  
25 (expression sequences) and translation of the coding sequences, such that the encoded polypeptide product is produced. Construction of such nucleic acids is well known in the art. For example, such nucleic acids can include a promoter, a transcription termination site  
30 (polyadenylation site in eukaryotic expression hosts), a ribosome binding site and, optionally, an enhancer for use in eukaryotic expression hosts and sequences necessary for replication of a vector.

### Antigens and Methods

The antigens of the present invention, and monoclonal or polyclonal antibodies raised or generated against these antigens, are useful as diagnostic reagents  
5 for detecting the presence of the fungus *H. capsulatum* in a sample, the presence of a previous or current infection by *H. capsulatum*, and for diagnosing histoplasmosis.

Numerous assay techniques based upon immunological reactions between antigens and antibodies may be  
10 performed with the antigens and antibodies of the invention to detect the presence of *H. capsulatum* in a sample, the presence of a previous or current infection by *H. capsulatum*, and for making a positive diagnosis of histoplasmosis, including the well-known enzyme-linked  
15 immunosorbent assays (ELISA), immunofluorescence assays (IFA), radioimmuno assays, immunoelectrophoresis, immunoblotting and the like.

Using any of the known assay techniques which are based upon immunological reactions, a previous or current  
20 *H. capsulatum* infection in a subject may be detected by the steps comprising: (a) contacting a fluid or tissue sample from the subject which contains antibodies with an isolated or recombinantly-produced antigen of the present invention; and (b) detecting the presence of binding  
25 between the antibodies and the antigen, the presence of binding indicating the presence of a previous or current *H. capsulatum* infection in the subject.

In these immunodiagnostic techniques, the antigen employed can be any of the isolated or recombinantly-produced polypeptides or polypeptide fragments described  
30 hereinabove. Because large quantities of polypeptides and polypeptide fragments can be safely produced by recombinant DNA techniques using nucleic acids described herein, and purified, it is preferable to use  
35 recombinantly-produced polypeptides and polypeptide

fragments in the immunodiagnostic techniques of the invention.

The nucleotide sequence which is complementary to the DNA nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1 encodes the *H. capsulatum* M antigen. Thus, the antigen employed in the immunoassay techniques described herein can be this protein, an antigenic polypeptide fragment of this protein, or any other antigenic polypeptide or polypeptide fragment encoded by nucleic acid which has a nucleotide sequence which is complementary to SEQ ID NO:1, or to a nucleic acid which has a nucleotide sequence which is substantially the same as the nucleotide sequence which is complementary to SEQ ID NO:1. It is already well established that the *H. capsulatum* M antigen is antigenic, and is specific for *H. capsulatum*. Fragments of the *H. capsulatum* M antigen may also possess one or more epitopes of the M antigen protein which are unique to *H. capsulatum*. These epitopes, and the polypeptides and polypeptide fragments containing them, can be readily determined by the well-known techniques of epitope mapping and conformational dependency analysis. Monoclonal antibodies directed against the M antigen may be utilized, as described in Zancopé-Oliveira et al., "Evaluation of Cation Exchange Chromatography for Purifying the M-glycoprotein Antigen from Histoplasmin," *J Med Vet Mycol* 31, 29-41 (1993), and Zancopé-Oliveira et al., "Immunochemical Analysis of Glycosidic Epitopes in the H and M Antigens from *Histoplasma capsulatum*," *Clinical and Diagnostic Laboratory Immunology*, 1: 563-568 (1994). The monoclonal antibodies can be applied in the enzyme-linked immunoelectrotransfer blot (western blot) method. In addition, partial digestion with proteinases can be utilized to fragment recombinant M antigen. The fragments can be purified by Fast Protein Liquid Chromatography (FPLC), and used in an intermediate gel to

inhibit the immune precipitation of M antigen by specific antiserum in 2 dimensional crossed rocket immunoelectrophoresis. Further, a phage display library with restriction endonuclease digested M antigen gene can  
5 be developed. The phages expressing peptides can be tested by replica plating for immunoreactivity by indirect enzyme immunoassay. By testing homologous antisera and monoclonal antibodies, and those obtained from heterologous fungi, one can determine which peptide  
10 fragments contain epitopes specific for *Histoplasma capsulatum*.

Polypeptides which may be employed in the immunodiagnostic assays and skin tests of the present invention are those encoded by the plus strands of the  
15 nucleic acids of the invention. Antigenic fragments of the polypeptides can be synthesized directly, or obtained by chemical or mechanical disruption of the fungus, or of the larger polypeptides. The antigenic polypeptides and polypeptide fragments of the present invention can also  
20 be recombinant proteins, polypeptides or fragments thereof, obtained by cloning nucleic acids encoding the proteins, polypeptides or fragments in an expression system capable of producing the antigenic proteins, polypeptides, or fragments thereof.

25 Using the deduced amino acid sequence of the *H. capsulatum* M antigen set forth in the Sequence Listing as SEQ ID NO:2, it is also possible to synthesize, using standard peptide synthesis techniques, polypeptide fragments chosen to be homologous to immunoreactive  
30 regions of the larger antigen, and to modify these fragments by inclusion, deletion or modification of particular amino acids residues in the sequences. The amino acid sequences of the antigens of the invention can contain an immunoreactive region attached to sequences  
35 designed to provide for some additional property, such as solubility. These amino acid sequences can also include

amino acid substitutions to provide for some additional property, such as to remove or add amino acids capable of disulfide bonding, to increase antigenicity and/or biolongevity, or to alter enzymatic activity. Thus, synthesis and purification of an extremely large number of polypeptides and polypeptide fragments derived from the *H. capsulatum* M antigen is possible. However, these polypeptides and polypeptide fragments need to have a bioactive property, such as antigenicity.

10       The isolated polypeptides and polypeptide fragments obtained or produced can be tested to determine their antigenicity (immunoreactivity), immunogenicity and specificity by the well-known methods discussed hereinabove. One example of an immunologic technique that may be used for the detection of current or previous infection by *H. capsulatum* utilizes monoclonal antibodies (MAbs) for detection of antibodies that specifically bind *H. capsulatum* M antigen. Briefly, sera or other body fluid from the subject is reacted with *H. capsulatum* M antigen bound to a substrate (e.g., an ELISA 96-well plate). After excess sera is thoroughly washed away, a labeled (e.g., enzyme-linked, fluorescent, radioactive, or the like) monoclonal antibody is then reacted with the previously reacted antigen-serum antibody complex. The amount of inhibition of monoclonal antibody binding is measured relative to a control (no patient serum antibody).

25       The isolated or recombinantly-produced antigens of the invention can also be used as skin test antigens in skin tests for histoplasmosis. These skin tests are performed in a manner known by those of skill in the art for this disease, and for other pulmonary diseases, such as tuberculosis. Generally, a small quantity (generally about 0.1 ml) of liquid, such as physiological saline, containing an antigen of the invention, such as the *H. capsulatum* M antigen, is injected intradermally beneath



the skin (on the forearm or other convenient location) of a patient, and the site of injection is observed at predetermined times, such as 24 and 48 hours post injection, for the presence of swelling of the skin. If no swelling of the skin at the injection site is observed, this indicates that the patient tested was not exposed to *H. capsulatum*. If swelling of the skin at the injection site is observed, this indicates that the patient tested has been exposed to *H. capsulatum*. Skin tests are usually observed, and the area of induration measured, at 24 hours, 48 hours and 72 hours after intradermal injection in the volar surface of the forearm. For general information concerning these skin tests, see Klimas, "Delayed Hypersensitivity Skin Testing," pp. 276-280, in Rose et al., Manual of Clinical Laboratory Immunology (5th ed., eds. American Society for Microbiology, Washington, 1996). For general information concerning the use of *H. capsulatum* glycoproteins in a skin test for the diagnosis of histoplasmosis, see Sprouse, "Determination of Molecular Weight, Isoelectric Point, and Glycoprotein Moiety for the Principal Skin Test-Reactive Component of Histoplasmin," *Infection and Immunity* 15, 263-271 (1977).

Prior to using the isolated or recombinantly-produced antigens in any immunodiagnostic assays or skin tests, it is preferable that the antigens be partially or fully deglycosylated by, for example, mild periodate oxidation with about 0.025 M sodium meta-periodate at about 4°C for about 4-8 hours in the dark, followed by reduction with sodium borohydride, and then an equimolar amount of glycerol.

#### Antibodies

An isolated antibody which binds with antigens of the present invention is also provided. The antibodies can be polyclonal or monoclonal, and should specifically

bind an epitope of an antigen which is specific to *H. capsulatum*. The term "bind" means the well-understood antigen-antibody interactions, or other nonrandom association with an antigen. "Specific binding" as used  
5 herein means an antibody that has a higher affinity for its target molecule (e.g., an antigen of the invention) than for non-target molecules (e.g., antigens of other closely-related fungi, or of other microorganisms).

Antibodies can be made by many well-known methods.  
10 See, for example, Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1988)). Briefly, an isolated or recombinantly-produced antigen can be injected into an animal in an amount, and in intervals, sufficient to  
15 elicit an immune response (i.e., the production of antibodies against the antigen). Antibodies can be obtained from the animal and purified directly by well-known methods. Alternatively, spleen cells can be obtained from the animal, and then fused with an immortal  
20 cell line and screened for monoclonal antibody secretion. The antibodies can be used to screen nucleic acid clone libraries for cells secreting the antigen. These positive clones can then be sequenced. The production of a murine monoclonal antibody (EC2-EC7) which is specific  
25 to the M antigen of *H. capsulatum* is described in Reiss et al., "Monoclonal Antibodies against the M-protein and Carbohydrate Antigens of Histoplasmin Characterized by the Enzyme-Linked Immunoelctrotransfer Blot Method," *Infection and Immunity*, 53, 540-546 (1986).

30 Specific examples of isolated antibody within the invention which specifically bind to *H. capsulatum* antigens include antibodies which specifically bind with an isolated or recombinantly-produced polypeptide encoded by a nucleic acid which has a nucleotide sequence which  
35 is complementary to SEQ ID NO:1, or to antigenic fragments thereof.

Using any of the known assay techniques which are based upon immunological reactions, a current *H. capsulatum* infection in a subject suspected of having an *H. capsulatum* infection may be detected by the steps comprising: (a) contacting a fluid or tissue sample from the subject which contains antigens with antibodies generated against an antigen of the present invention; and (b) detecting the presence of binding between the antigens and the antibodies, the presence of binding indicating the presence of a current *H. capsulatum* infection in the subject.

General information concerning the reactions of antibodies to antigens of *H. capsulatum* is present in Kumar et al., "Cross-Reacting Human and Rabbit Antibodies to Antigens of *Histoplasma capsulatum*, *Candida Albicans* and *Saccharomyces Cerevisiae*," *Infect. Immun.* 48:806-812 (1985); Reiss et al., "Monoclonal Antibodies Against the M Protein and Carbohydrate Antigens of Histoplasmin Characterized by the Enzyme-Linked Immunoelctrotransfer Blot Method," *supra.*; and Harris, "Characterization of Anigenic Determinants in Histoplasmin that Stimulate *Histoplasma Capsulatum*-Reactive T Cells in Vitro," *Infection and Immunity* 56, 2343-2349 (1988).

### Kits

The present invention also provides a kit for detecting a previous or current *H. capsulatum* infection in a sample, or for diagnosing histoplasmosis. Preferably, the kit will contain one or more of the isolated nucleic acids, isolated or recombinantly-produced antigens, or isolated antibodies of the invention, and instructions describing the use of the nucleic acids, antigens or antibodies in the detection of a previous or current *H. capsulatum* infection, or in the diagnosis of histoplasmosis.

### Vaccines

The isolated nucleic acids and isolated or recombinantly-produced antigens of the present invention may be used as the active component in an  
5 immunogenically-effective amount (an amount which is effective to stimulate the production of antibodies against the nucleic acids or antigens in the particular subject being vaccinated) in a vaccine for the prevention or treatment of histoplasmosis along with a  
10 pharmaceutically-acceptable carrier for the nucleic acids or antigens to provide protective resistance against *H. capsulatum*. Such a vaccine would be particularly useful for individuals who are at a high risk for contracting histoplasmosis, such as individuals who explore caves  
15 where birds and/or bats may be present, and individuals who deconstruct vacant buildings, which be inhabited by birds and/or bats.

Active immunization can be achieved through natural infection with an organism or virus, or artificially by  
20 vaccination. (See, for example, Kuby, Immunology (W.H. Freeman and Co., New York (1992)).) It is also contemplated that immunization against disease caused by *H. capsulatum* can be achieved by a "naked" DNA vaccine approach. Briefly, DNA constructs containing promoter  
25 sequences upstream of *H. capsulatum* M antigen coding sequences can be injected into muscle tissue or administered via the mucosa and result in expression of antigens that induce a protective immune response.

An immunogenically-effective amount of the nucleic acids or antigens of the invention will generally range  
30 from about 100 nanograms to about 1 microgram of the nucleic acids, and from about 10 to about 100 micrograms of the antigens. Immunogenically-effective amounts of the vaccine, nucleic acid or antigen can be determined  
35 using standard procedures. Briefly, various concentrations of the nucleic acid or antigen are

prepared and administered to an animal, and then the immunological response (e.g., the production of antibodies or cell mediated immunity) of the animal to each concentration is determined. The amounts of nucleic acid or antigen administered depend on the subject, e.g. a human or an animal, the condition of the subject, the size of the subject, etc. Thereafter, the animal so inoculated with the nucleic acid or antigen can be exposed to *H. capsulatum* to test the potential vaccine effect (protective immunogenicity) of the specific nucleic acid or antigen. The specificity of the nucleic acid or antigen can be ascertained by testing sera, other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely-related fungi, or other microorganisms.

The pharmaceutically-acceptable carrier which may be employed in the vaccines can comprise saline or other suitable carriers. See, for example, Arnon, R. (Ed.) Synthetic Vaccines (CRC Press, Inc., Boca Raton, Florida (1987)). By "pharmaceutically-acceptable" is meant a material that may be administered to a subject along with a selected nucleic acid or antigen without causing any undesirable biological effects, or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier will depend upon the method of administration and choice of adjuvant, if one is used. An adjuvant can also be a part of the carrier of the vaccine, in which case it can be selected by standard criteria based upon the nucleic acid or antigen used, the mode of administration and the subject. Methods of administration can be by oral or sublingual means, or by injection, depending on the particular vaccine used, and the subject to whom it is administered.

The vaccine can be used as a prophylactic or a therapeutic modality. Thus, the invention contemplates

methods of preventing or treating infection from *H. capsulatum*, and the associated diseases, by administering the vaccine to a subject.

5       The following Example describes and illustrates the methods for the cloning and sequencing of the *H. capsulatum* M antigen gene. This Example is intended to be merely illustrative of the present invention, and not limiting thereof in either scope or spirit. Those of skill in the art will readily understand that variations  
10 of the reagents used in, and the conditions and processes of, the procedures described in this Example can be used to clone and sequence nucleic acids which are specific to *H. capsulatum*.

15       All materials and equipment employed in the Example, and generally employed to make and use the nucleic acids, polypeptides, vaccines and kits of the present invention, are commercially-available. Sources for these materials and equipment are set forth in the Example, or are known by those of skill in the art.

EXAMPLECloning and Sequencing of  
the *H. capsulatum* Gene Encoding the M Antigen

In these experiments, the gene encoding the M  
5 antigen of *H. capsulatum* var. *capsulatum* (anamorph name,  
but also known by the teleomorph name *Ajellomyces*  
*capsulatus*) Centers for Disease Control and Prevention  
(CDC) strain 6623, which is deposited with the ATCC under  
Accession Number ATCC 26320, was cloned and sequenced.  
10 Semi-purified M. protein was transferred to PVDF  
membranes. The eluted protein was sequenced directly,  
and cleaved with various proteinases, and the internal  
peptides were sequenced by microbore HPLC. Although the  
NH<sub>2</sub> terminus was blocked, several internal amino acid  
15 sequences were obtained. A homology search through a  
protein data base revealed significant similarity of  
these amino acid sequences to both eukaryotic and  
prokaryotic catalases. This degree of conservation  
facilitated peptide alignments. Degenerate  
20 oligonucleotides were constructed in the proper  
orientation for polymerase chain reactions (PCR). The  
amino acid sequence derived from the resulting amplicon  
confirmed that it encoded a region of the M antigen gene.  
This probe was used to screen an *H. capsulatum* genomic  
25 library, and a 4.0 kb fragment containing the entire M  
antigen gene was cloned and sequenced by the dideoxy  
chain termination method of Sanger et al., "DNA  
Sequencing with Chain-Terminating Inhibitors," *Proc.*  
*Natl. Acad. Sci USA* 74, 5463-5467 (1977). This gene was  
30 found to contain five introns, as determined by sequence  
analysis of cDNA obtained by reverse transcription  
polymerase chain reaction, and to be homologous with  
other members of the catalase family. The nucleotide  
sequence (DNA) for this M antigen gene, which contains

3862 nucleotides, is set forth in the Sequence Listing as SEQ ID NO:1. For the mature protein (not including a sixteen amino acid leader sequence), the open reading frame starts at base pair number 566 of the genomic clone, and stops at base pair number 2812 thereof. Introns are present in the nucleotide sequence between six exons, which are present at base pair numbers 566-793, 852-1077, 1168-1583, 1706-1870, 1950-2124 and 2208-3121. The sequence of the clone containing the M antigen gene has been deposited in GenBank under accession number AF0 26268.

#### **Materials and Methods**

**Strains, plasmids and cultures conditions.** Yeast-phase cells of *H. capsulatum* strain 6623 (ATCC 26320) were grown at 37°C in Pine's Liquid Medium for 48 hours to late log phase. *E.coli* strain q358 was used as the host for the bacteriophage 1 Gem11, and *E.coli* INV aF' (Invitrogen Co., Carlsbad, CA) was used as the recipient for the subcloning vector pBluescript SK (Stratagene, La Jolla, CA).

**Purification of the M antigen.** M antigen was purified by tandem cation exchange chromatography in columns of CM Sepharose CL-6B from histoplasmin, as described by Zancopé-Oliveira et al., "Evaluation of Cation Exchange Chromatography for the Isolation of M Glycoprotein from Histoplasmin," supra.

**Amino acid sequence of M antigen.** Samples of M antigen were electrophoresed on 10% SDS-PAGE, and transferred for 1 hour at 400 mA to polyvinylidene difluoride membranes (Immobilon-P, Milipore Corp., Bedford, Mass.) in 25 mM Tris, 192 mM glycine, and methanol (20%[vol/vol]). The membrane was washed several times with 1 mM DTT, stained with Ponceau S and destained



with 10% aldehyde-free acetic acid-1 mM DTT. Several washings with 1 mM DTT were made to remove the acetic acid. The band was identified by its molecular weight, and its identity was confirmed by immunoblotting. The  
5 band corresponding to M antigen (200 pmol/protein band) bound to the membrane was excised, and submitted to Edman degradation without any prior modification. To obtain the internal sequences, the band was digested in situ with lysyl endopeptidase (Boehringer Mannheim,  
10 Indianapolis, IN), and peptides were purified using microbore reverse-phase high-performance liquid chromatography (HPLC) on reverse phase C18 silica. All amino acid sequences were obtained using ABI sequencers (models 477A Protein Sequencer or Procise, Applied  
15 Biosystems, Foster City, CA) which utilize pulse-liquid chemistry.

**DNA isolation.** Yeast cells grown in 50 ml of Pine's broth were harvested by filtration on 0.45  $\mu$ m porosity membrane (Nalgene), washed 3 times with deionized H<sub>2</sub>O and  
20 blotted to remove excess moisture. Cells were placed in a sterile mortar with approximately 1 g glass beads (0.5 mm), and liquid nitrogen, and were ground to a fine powder. The powder was resuspended in 20 ml of TE Buffer, pH 8.0 (10 mM Tris-1 mM EDTA), and DNA was  
25 extracted with phenol, ethanol precipitated, and dried and redissolved in 0.05 M TE. The RNA was removed by the addition of RNase (10  $\mu$ g/ml final concentration) (Boehringer Mannheim) at 37°C for 1 hour, followed by proteinase K treatment (50  $\mu$ g/ml) (Sigma Chemical Co.,  
30 St. Louis, MO) for an additional 1 hour at 37°C. The DNA was subjected again to phenol extraction, and EtOH precipitation, and redissolved in TE.

**Generation of M DNA probe by PCR.** *H. capsulatum* genomic DNA was used to amplify a DNA fragment encoding  
35 an internal portion of the M protein by PCR. Degenerated

oligonucleotides primers (1  $\mu$ M) were designed on the basis of two of six internal peptides (V22 and V18) derived from the amino acid sequence of the M antigen, which are set forth in Table 1, because the NH<sub>2</sub> terminus appeared to be blocked:

**Table 1**

Amino Acid Sequences of NH<sub>2</sub>-Terminus and Lysyl Endopeptidase-Digested Fragments of the M Antigen

	<u>Origin</u>	<u>Amino Acid Sequence</u>
10	<u>NH<sub>2</sub> terminus</u>	S D P T D Q F L (SEQ ID NO:3)
<u>Internal Sequences</u>		
	2642-m1947/19	D F I F R Q K I Q H F D H E R (SEQ ID NO:4)
	5070-m1941/20	T L Q G R A G L V (SEQ ID NO:5)
15	V22-m1947/20	A Q A L G G K N P D F H R Q D L (SEQ ID NO:6)
	V21-m1947/12	S G R Y P E (SEQ ID NO:7)
	V16-m1941/21	F D F D L L D P T K (SEQ ID NO:8)
	V18-m1941/23	I I P E E L V P F T P I G K (SEQ ID NO:9)

20 The sense primer M4F [5'-AA(AG)AA(CT)CC(AGC)GA(CT)TT(CT)-3',  
SEQ ID NO:10] was a 15-mer with 48-fold degeneracy,  
and the antisense primer  
M8R [5'-TT(AGCT)CC(AGT)AT(AGCT)GT(AG)AA-3', SEQ ID  
NO:11] was a 14-mer with 96-fold degeneracy. PCR was  
25 carried out in a total volume of 100  $\mu$ l containing 100 ng

of DNA as template, 100 M each of dNTP, 1 M of each oligonucleotide primer, and 10X PCR Buffer containing 500 mM KCl, 100 mM Tris-HCl, pH 8.3, 25 mM MgCl<sub>2</sub>, and 2.5 U of Taq polymerase (Boehringer Mannheim). The  
5 amplification conditions consisted of a denaturation at 95°C for 5 minutes followed for 35 cycles of the succeeding steps: denaturation at 95°C for 5 minutes, annealing at 50°C for 1 minute, and extension at 72°C for 1 minute. A final elongation was done at 72°C for 5  
10 minutes. A 300-bp PCR product was subcloned into the pCRII vector using the TA cloning kit (Invitrogen, San Diego, Calif.), and using procedures recommended by the vendor, and sequenced using a dye-labeled terminator and automated sequencer (Applied Biosystems).

15        Screening of an *H. capsulatum* genomic library. The 300-bp amplicon was labeled with [-<sup>32</sup>P]dCTP by High Prime DNA Labeling Mix (Boehringer Mannheim), purified in a DEAE column (NACS Prepac Convertible - BRL Life  
20 Technologies, Inc.), and used for screening the genomic library, derived from DNA partially digested with Sau3A1 and cloned into lGem11 via the Xho 1 half-site. An *E. coli* q358 strain bacterium infected with the genomic library, was replica plated onto nitrocellulose membranes. Plaques were lysed, and then heat fixed.  
25 Filters were hybridized with <sup>32</sup>P-labeled probe. Twelve positive colonies were picked, and rescreened as large plaques. Two strongly positive plaques were purified and mapped by Southern analysis. These clones were digested with BamH1 and one fragment of 4.0 kb was obtained.

30        Gene sequence analysis: The 4.0 kb fragment was subcloned into pBluescript II KS, and sequenced by the strategy of ~~primer walking~~ using the dideoxy chain termination method. Oligonucleotides of 22-mer were synthesized on the basis of DNA sequence and applied to

initiate the sequence reaction. The clone was sequenced in both directions. To determine the sites of putative introns, 5 µg of RNA was reverse transcribed using oligo-dT to initiate the cDNA reaction. The first strand of cDNA was amplified with a sense primer located at the start site of the mature protein: the sequence of this primer was 5'-CGGAATCCTCCGACCTACGGA-3' (SEQ ID NO:12). The antisense primer was 5'-ACCAAGCTTCTATCCAACGGGAACCGA-3' (SEQ ID NO:13). A 5'*Eco*RI site (underlined) was added to the sense primer, and a *Hind*III site (underlined) was added to the antisense primer to facilitate cloning in pBluescript SK-. PCR was performed for 35 cycles of 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 2 minutes with 5 U of Vent polymerase (New England Biolabs, Beverly, Mass.). The PCR product was digested with *Eco*RI and *Hind*III and cloned into pBluescript SK-, restriction mapped and sequenced in its entirety. The gene encoding the M antigen was deposited in GenBank, and its accession number is AF026268.

## 20 Results

M antigen amino acid sequencing. Peptides sequences of the M antigen were determined after digestion of purified M glycoprotein with lysyl endopeptidase, and purification using high-performance liquid chromatography (HPLC). Undigested antigen and internal peptides were sequenced by Edman degradation. The amino acid sequences of the NH<sub>2</sub> terminus and 6 internal peptides are shown in Table 1. The amino acid sequences of two internal

peptides, V22 and V18, of the M protein (Table 1) showed 66-73% of identity with sequences of catalases of *Schizosaccharomyces pombe* (gpD55675 YSPC\_1) and *Aspergillus niger* (gpZ23138 ANCATRGNA\_1).

Cloning and sequencing of the M gene. The significant degree of homology of the two internal peptides V22 and V18 to fungi catalases suggested a certain arrangement in the protein. Considering their positions, two degenerate oligonucleotides (sense primer M4F and antisense primer M8R) were designed, based upon the two internal peptides V22 and V18, respectively, and used in a PCR reaction to amplify a 320 base pair fragment of *H. capsulatum* genomic DNA. A 300 base pair PCR product was achieved using M4F and M8R as primers, and confirmed by Southern blot to represent a unique gene of *H. capsulatum*. Sequence analysis of this 300 base pair amplicon obtained by the dideoxy chain terminator method enclosed the two native internal peptides, confirming that the PCR product encoded a region of the gene encoding the M antigen.

To isolate the entire gene encoding the M antigen, the 300 base pair PCR fragment was gel purified in 1% agarose, and used to screen an *H. capsulatum* genomic DNA library. A *Bam*HI genomic fragment of 4.0 kb carrying the gene encoding the M antigen was isolated and characterized. This fragment was subcloned into pBluescript II KS, and was sequenced in its entirety in both directions. SEQ ID NO:1 shows the complete nucleotide sequence of the *H. capsulatum* gene encoding the M antigen, and SEQ ID NO:2 shows the deduced amino acid sequence, which consisted of 707 amino acid residues (including a sixteen amino acid leader sequence) with an estimated molecular weight of about 78,172 Da.

The coding region of the M antigen gene is set forth in SEQ ID NO:1. It is interrupted by 5 introns, which begin and end at the base pair numbers 794-851, 1078-1167, 1584-1705, 1871-1949 and 2125-2207, with the

5' and 3' extremities presenting the GT/AG consensus. The 5'-565 base pair flanking sequence of this gene (the 565-base pair sequence directly preceding the first exon (first coding sequence)) exhibited similarity with the promoter regions of eucaryotic genes. A TATA element is present at base pair position 318, and a T+C-rich pyrimidine block is found downstream at base pair position 365. The CAA motif is found twice upstream of the T+C block at base pair positions 34 and 341. The 3'-region downstream from the M antigen gene open reading frame contains a pentanucleotide (5'-AAATA-3') at base pair position 3134, 19 nucleotides downstream from the termination codon. This sequence is similar to the polyadenylation consensus sequence described in eukaryotic organisms. It may play a role in the termination of transcription, processing, and addition of poly(A) at the 3'-terminus.

**Protein structure.** Sequencing of the N-terminus of the native protein revealed that the first residue of the mature protein is the serine residue at base pair position 566. The mature protein is 691 amino acids with a predicted size of 76,398 Da. Therefore, the expected M antigen gene has a leader peptide composed of 16 amino acids (the 16 amino acids which precede the serine residue at base pair number 566, and which begin with methionine) resulting in an amino acid sequence of 707 amino acids. Five potential N-glycosylation sites (NXT or NXS) were predicted.

**Comparison of the amino acid sequence of the M antigen gene with k sequences.** The earlier data base results showing that two peptides sequences of M protein had 66-73% of identity with sequences of catalases of

*Schizosaccharomyces pombe* (gpD55675 YSPC\_1) and *Aspergillus niger* (gpZ23138 ANCATRGNA\_1) suggested that the M antigen could be a catalase. Comparison of the M deduced amino acid sequence with known fungal catalases from *Aspergillus fumigatus* (GenBank accession number u87850), *Eimericella nidulans* (GenBank accession number u80672), *Aspergillus niger* (GenBank accession number l15474), and *Saccharomyces cerevisiae* (GenBank accession number x13028), using a Genetics Computer Group, Inc., computer program, demonstrated 61.2, 60.4, 53.2 and 21.7% of similarity at the amino acid level, respectively. The M antigen amino acid sequence can be divided into parts of high and low homology with these other amino acid sequences, which may suggest functional domains.

15        Copy number of M gene. Southern blot of *H. capsulatum* genomic DNA digested with various restriction enzymes was probed with the 320-base pair PCR product in order to evaluate the genomic organization of the M antigen gene. A single hybridized band of 4.0 kb was  
20        seen with the *Bam*HI-digested genomic DNA, which corresponded to the size of the lGem11 purified inserts. The hybridization profile of the other fragments manifested only a single band, suggesting that a single copy or few copies of the M antigen gene could occur in  
25        the genome.

30        The foregoing Example is provided to enable one of ordinary skill in the art to practice the present invention. This example is merely illustrative, however, and should not be read as limiting the scope of the invention as it is claimed in the appended claims.

While the present invention has been described herein with some specificity, and with reference to

certain preferred embodiments thereof, those of ordinary skill in the art will recognize numerous variations, modifications and substitutions of that which has been described which can be made, and which are within the scope and spirit of the invention. It is intended that all of these modifications and variations be within the scope of the present invention as described and claimed herein, and that the invention be limited only by the scope of the claims which follow, and that such claims be interpreted as broadly as is reasonable.

Specific nucleic acids, antigens, antibodies, vaccines, methods and kits within the scope of the invention include, but are not limited to, the nucleic acids, antigens, antibodies, vaccines, methods and kits described herein. Contemplated equivalents of the nucleic acids, antigens, antibodies, vaccines, methods and kits described herein include nucleic acids, antigens, antibodies, vaccines, methods and kits which otherwise correspond thereto, and which have the same general properties thereof, wherein one or more simple variations are made which do not adversely affect the function of the nucleic acids, antigens, antibodies, vaccines, methods and kits as described herein.

The Sequence Listing which is present herein uses the symbols for bases and amino acids which are described in §2423 of the U.S. Patent and Trademark Office Manual of Patent Examining Procedure, in which R represents A or G, Y represents C or T/U and V represent A or C or G.

Throughout this application, various patents, publications, books, nucleic acid and amino acid sequences, and computer programs have been cited. The



entireties of each of these patents, publications, books, nucleic acid and amino acid sequences, and computer programs are hereby incorporated by reference herein into this application.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid specific to *Histoplasma capsulatum* comprising:
  - a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;
  - a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;
  - a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;
  - a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;
  - a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;
  - a fragment of a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;
  - a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1; or
  - a fragment of a nucleic acid having a nucleotide sequence which is substantially the same as a nucleic acid which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;
- wherein the nucleic acid does not contain the nucleotide sequences 5'CGAAGTCGAGGCTTTCAGCATG3',  
5 TATTAGCTCTAGAATTACCACGGGTATCCAAGTAGTAAGG3 ,  
5 CCCC GAAGGGCATTGGTTTTTTTATCTAATAAATACACCCC3 , or  
nucleotide sequences complementary thereto,
- and wherein the nucleic acid is not a nucleic acid

consisting essentially of between 10 and 100 nucleotides which is able to form a hybrid at 60°C with a nucleotide polymer having a nucleotide sequence of

5' CGAAGTCGAGGCTTTCAGCATG3' , 5' CATGCTGAAAGCCTCGACTTCG3 ,  
5 5' CAUGCUGAAAGCCUCGACUUCG3' or 5' CGAAGUCGAGGCUUUCAGCAUG3 .

2. The nucleic acid of Claim 1, wherein the nucleic acid has the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

3. The nucleic acid of Claim 1, wherein the nucleic  
10 acid has a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

4. The nucleic acid of Claim 1, wherein the nucleic  
15 acid has a nucleotide sequence which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

5. The nucleic acid of Claim 1, wherein the nucleic  
20 acid has a nucleotide sequence which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

6. The nucleic acid of Claim 1, wherein the nucleic  
25 acid is a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

7. The nucleic acid of Claim 1, wherein the nucleic  
30 acid is a fragment of a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

8. The nucleic acid of Claim 1, wherein the nucleic acid is a fragment of a nucleic acid having a nucleotide sequence which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

9. The nucleic acid of Claim 1, wherein the nucleic acid is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

10. An isolated or recombinantly-produced antigen specific to *Histoplasma capsulatum* comprising:

a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1; or

a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

11. The antigen of Claim 10, wherein the antigen is a polypeptide encoded by a nucleic acid having a

nucleotide sequence which is complementary to the  
nucleotide sequence set forth in the Sequence Listing as  
SEQ ID NO:1.

12. The antigen of Claim 11, wherein the antigen  
5 has an amino acid sequence as set forth in the Sequence  
Listing as SEQ ID NO:2.

13. The antigen of Claim 10, wherein the antigen is  
a fragment of a polypeptide encoded by a nucleic acid  
having a nucleotide sequence which is complementary to  
10 the nucleotide sequence set forth in the Sequence Listing  
as SEQ ID NO:1.

14. The antigen of Claim 10, wherein the antigen is  
a polypeptide encoded by a nucleic acid which is  
substantially the same as a nucleic acid having a  
15 nucleotide sequence which is complementary to the  
nucleotide sequence set forth in the Sequence Listing as  
SEQ ID NO:1.

15. The antigen of Claim 10, wherein the antigen is  
a fragment of a polypeptide encoded by a nucleic acid  
20 which is substantially the same as a nucleic acid having  
a nucleotide sequence which is complementary to the  
nucleotide sequence set forth in the Sequence Listing as  
SEQ ID NO:1.

16. A vector comprising a nucleic acid specific to  
25 *Histoplasma capsulatum*, wherein the nucleic acid:  
has a nucleotide sequence as set forth in the  
Sequence Listing as SEQ ID NO:1;  
has a nucleotide sequence which is substantially the  
same as a nucleotide sequence as set forth in the  
30 Sequence Listing as SEQ ID NO:1;  
is a fragment of a nucleic acid having a nucleotide

sequence as set forth in the Sequence Listing as SEQ ID NO:1; or

5 is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1; and wherein the vector is suitable for expressing the nucleic acid.

10 17. The vector of Claim 16, wherein the nucleic acid has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

15 18. The vector of Claim 16, wherein the nucleic acid has a nucleotide sequence which is substantially the same as a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

19. The vector of Claim 16, wherein the nucleic acid is a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

20 20. The vector of Claim 16, wherein the nucleic acid is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

21. A method for detecting a previous or current *Histoplasma capsulatum* infection in a subject, comprising:

5 (a) contacting a fluid or tissue sample from the subject which contains antibodies with an isolated or recombinantly-produced antigen which is specific to *Histoplasma capsulatum*; and

10 (b) detecting the presence of binding between the antibodies and the antigen, the presence of binding indicating the presence of a previous or current *Histoplasma capsulatum* infection in a subject, wherein the antigen is:

a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the  
15 nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

20 a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as  
25 SEQ ID NO:1; or

a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing  
30 as SEQ ID NO:1.

22. The method of Claim 21, wherein the antigen is a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence as set forth in the Sequence Listing  
35 as SEQ ID NO:1.

23. The method of Claim 22, wherein the antigen has an amino acid sequence as set forth in the Sequence Listing as SEQ ID NO:2.

5 24. The method of Claim 21, wherein the antigen is a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

10 25. The method of Claim 21, wherein the antigen is a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

15 26. The method of Claim 21, wherein the antigen is a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as  
20 SEQ ID NO:1.

27. An isolated antibody produced against an antigen of Claim 10.

28. A kit for detecting a previous or current *Histoplasma capsulatum* infection in a sample comprising:

25 (a) an isolated nucleic acid of Claim 1, an isolated or recombinantly-produced antigen of Claim 10 or an isolated antibody of Claim 27; and

(b) instructions describing the use of the nucleic acid, antigen or antibody in the detection of a previous  
30 or current *Histoplasma capsulatum* infection.



29. The kit of Claim 28, wherein the kit contains an antigen, and the antigen is a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

30. The kit of Claim 28, wherein the kit contains an antigen, and the antigen is a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

31. A host for expressing a polypeptide specific to *Histoplasma capsulatum* comprising a vector containing a nucleic acid, wherein the vector is suitable for expressing the nucleic acid, and wherein the nucleic acid:

has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;

has a nucleotide sequence which is substantially the same as a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1;

is a fragment of a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1; or

is a fragment of a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

32. The host of claim 31, wherein the nucleic acid has a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

33. The host of Claim 31, wherein the nucleic acid is substantially the same as a nucleic acid having a nucleotide sequence as set forth in the Sequence Listing as SEQ ID NO:1.

5           34. A method for detecting a past exposure to the fungus *Histoplasma capsulatum* comprising:

          (a) injecting intradermally in the skin of a patient an effective amount of an isolated or recombinantly-produced antigen which is specific to  
10 *Histoplasma capsulatum*; and

          (b) observing the skin at the injection site at a predetermined time after injection for a presence of swelling of the skin, the presence of swelling of the skin indicating a past exposure by the patient to the  
15 fungus *Histoplasma capsulatum*,  
wherein the polypeptide is:

          a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as  
20 SEQ ID NO:1;

          an antigenic fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

25           a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1; or

30           an antigenic fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

35. The method of Claim 34, wherein the antigen is a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence as set forth in the Sequence Listing  
5 as SEQ ID NO:1.

36. The method of Claim 34, wherein the antigen is an antigenic fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the  
10 Sequence Listing as SEQ ID NO:1.

37. The method of Claim 34, wherein the antigen is a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the  
15 nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

38. The method of Claim 34, wherein the antigen is a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having  
20 a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1.

39. A vaccine for the prevention of histoplasmosis comprising:  
25 (a) an effective amount of a nucleic acid of claim 1 or an isolated or recombinantly-produced antigen of Claim 10; and  
(b) a pharmaceutically-acceptable carrier.

40. A method for detecting a current *H. capsulatum* infection in a subject suspected of having an *H. capsulatum* infection comprising:

5 (a) contacting a fluid or tissue sample from the subject which contains antigens with antibodies generated against an antigen which contains an epitope which is unique to *H. capsulatum*; and

(b) detecting the presence of binding between the antigens and the antibodies, the presence of binding  
10 indicating the presence of a current *H. capsulatum* infection in the subject,

wherein the antigen is:

a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary to the  
15 nucleotide sequence set forth in the Sequence Listing as SEQ ID NO:1;

a fragment of a polypeptide encoded by a nucleic acid having a nucleotide sequence which is complementary  
to the nucleotide sequence set forth in the Sequence  
20 Listing as SEQ ID NO:1;

a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing as  
25 SEQ ID NO:1; or

a fragment of a polypeptide encoded by a nucleic acid which is substantially the same as a nucleic acid having a nucleotide sequence which is complementary to the nucleotide sequence set forth in the Sequence Listing  
30 as SEQ ID NO:1.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Zancopé-Oliveira, Rosely M.  
et al.,
- 5 (ii) TITLE OF INVENTION: Nucleic Acids of the M Antigen  
Gene of Histoplasma Capsulatum, Isolated and  
Recombinantly-Produced  
Antigens, Vaccines and Antibodies, Method
- (iii) NUMBER OF SEQUENCES: 13
- 10 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Fitch, Even, Tabin & Flannery  
(B) STREET: 135 South LaSalle Street, Suite 900  
(C) CITY: Chicago  
(D) STATE: IL  
15 (E) COUNTRY: USA  
(F) ZIP: 60603-4277
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
20 (C) OPERATING SYSTEM: Windows  
(D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
25 (B) FILING DATE:  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:  
30 (A) NAME: Kaba, Richard A  
(B) REGISTRATION NUMBER: 30,562  
(C) REFERENCE/DOCKET NUMBER: 6314/62527
- (ix) TELECOMMUNICATION INFORMATION:  
35 (A) TELEPHONE: 312-372-7842  
(B) TELEFAX: 312-372-7848  
(C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
40 (A) LENGTH: 3862 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: Genomic DNA  
45 (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Histoplasma capsulatum  
(B) STRAIN: var. capsulatum

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATCCTGCT GGCTCCGATA ACTTTGCTTT ATCCAAGGGT CTCGGCGAAT GCCAGGTGCC 60  
ATCGATCTAT ATTTGAAGT TTATCACCTC AATGGCTTCA CCCCATGACG CACCTTTTAT 120  
50 TTTTATTTTC ATTCATCTTC TCTGTGGCAA ACATGCAGGT ATGCGAGCTC TGGACCCTGG 180

	GGTGTGGCCC	TTGATGCATA	TGGTTTATTT	ATAGCCGCCC	GGAAGCCCTG	GCCTGTAAAA	240
	TTTTGGACCT	CCTCCCGCCA	TTCTTTCCAA	ACTTCGTGCG	TCCGTTTCCC	ATTTCCCCCC	300
	TCCCATTTTG	GGTTCCCTAT	AGGCCACTGC	GTGCTCCACT	CAAGAAGGGT	CCCAGTCAAT	360
	TTGGTCCCTA	CCCTCTCCAA	CACTATCTGC	ATATGTAATA	TATATCGATA	TCTAACTGCC	420
5	ATTGATTATT	TGTCTCTTTC	AGCATCTTTT	TGTCTCGAGC	AAGCTTACTC	CACGTTCAAT	480
	TCAGGGGGTA	AAAATGCGGT	CGCTCAAGCT	TATACTCGCC	TCGGCGGGTG	TTGTTTCTGC	540
	AGCCTGTCCC	TACATGTCAG	GGGAGATGCC	TAGCGGTCAG	AAAGGCCCCC	TCGATCGCCG	600
	CCATGACACT	CTCTCCGACC	CTACGGACCA	GTTTCTTAGC	AAGTTTTACA	TTGACGATGA	660
	ACAGTCGGTG	CTAACAACGG	ACGTGGGTGG	TCCCATCGAG	GACCAACACA	GCCTGAAGGC	720
10	TGGAATAGA	GGCCCAACTC	TACTTGAGGA	TTTTATCTTC	CGCCAGAAGA	TTCAACACTT	780
	TGATCATGAG	AGGGTATGTA	GATACAAAAT	ATGTGACCGT	GTTGCAAATC	CGCTAATTCA	840
	ATTTTACGCA	GGTTCCTGAG	CGCGCCGTCC	ATGCTCGAGG	AGCTGGTGCC	CATGGCGTAT	900
	TCACATCTTA	TAATAACTGG	TCGAATATCA	CAGCCGCATC	CTTCTTGAAC	GCGGCAGGAA	960
	AGCAGACACC	AGTATTCGTG	CGGTTTCTTA	CAGTCGCTGG	TAGCAGAGGC	AGTGTGTACT	1020
15	CTGCTCGCGA	TATCCACGGA	TTTGGCAGCC	GTCTGTATAC	CGATGAAGGC	AATTTTGGTA	1080
	AGCATTATAT	CGTGGTAGTC	ATACTCATAA	CAGCACAAAC	AATATGAATA	CAAAACCAGG	1140
	ACCTAGGCTG	ACTACTCGGC	AATGTAGATA	TGCTCGGAAA	CAACGTTCCA	GTCTTCTTCA	1200
	TTCAGGACGC	TATTCAATTC	CCTGATTTGA	TTACGCTGT	CAAGCCGCAA	CCAGACAGTG	1260
	AAATTCCCCA	GGCTGCAACT	GCACATGATA	CGGCATGGGA	TTTCTCAGC	CAGCAGCCCA	1320
20	GCTCATTGCA	TGCCCTCTTC	TGGGCAATGT	CAGGACATGG	AATCCCTCGC	TCAATGCGCT	1380
	ATGTTGATGG	GTGGGGCGTC	CATACCTTCC	GACTTGTAC	CGACGAGGGC	AACTCGACCT	1440
	TGGTCAAGTT	TCGCTGGAAG	ACCCTCCAAG	GAAGAGCGGG	CCTGGTATGG	GAAGAGGCAC	1500
	AGGCTCTTGG	CGGAAAGAAT	CCCGACTTCC	ATCGACAAGA	CCTCTGGGAT	GCCATTGAAT	1560
	CTGGAAGGTA	CCCTGAGTGG	GAGGTAAGAT	ATGATTCCCC	CAAATCATT	GTCTGACAG	1620
25	TGTTTCTCTG	CTCTGTGCGT	TGCTCTTTTC	GTCTTTTCT	ATATCTTCAA	CTAAGACTGA	1680
	CTTTATATAC	GTTTTACTCA	TATAGCTGGG	CTTTCAATTG	GTGAATGAAG	CAGATCAATC	1740
	CAAGTTTGTAT	TTGATCCAC	CAAAATCATC	CCAGAAGAAC	TTGTTCTTTT	1800	
	CACCCCAATC	GGAAAAATGG	TCTTGAACCG	AAACCCAAAA	AGTTATTTTG	CCGAAACTGA	1860
	GCAGATCATG	GTGGGTCCAC	CCCCATATATA	TTTGGAAATAT	GAATACATGT	ATAGCTAGAT	1920
30	GAAGCGTATA	TCTAAATATA	TTTCCACAGT	TCCAACAGG	TCATGTAGTT	CGCGGAATCG	1980
	ATTTACGGA	TGACCCTTTG	CTTCAGGGCC	GCTTGTACTC	CTACCTTGAC	ACTCAATTGA	2040
	ATCGCCATGG	AGGTCCCAAC	TTTCAGCAAC	TGCCGATCAA	CAGACCCCGC	ATCCCATTC	2100
	ATAACAACAA	TCGCGACGGT	GCTGGTAAGC	TACTTCTCAC	CTACCATGTC	AACTTCCATC	2160
35	TTGACCCAAT	CGATTTGTAT	AGAGTATTAA	CATCCCCGTC	TGCACAGGAC	AAATGTTTAT	2220
	CCCTCTAAAC	ACGGCCGCGAT	ATACACCCAA	CTCAATGAGC	AACGGATTCC	CACAACAAGC	2280
	CAACCGGACC	CATAACAGAG	GATTCTTCAC	CGCACCTGGG	CGTATGGTAA	ATGGACCACT	2340
	AGTGCGCGAG	CTCAGCCCGA	GCTTCAACGA	CGTCTGGTCC	CAACCGCGTC	TCTTCTACAA	2400
	CTCACTCAGC	GTCTTCGAGA	AGCAATTCCT	CGTCAACGCC	ATGCGCTTCG	AAAACCTCCA	2460
40	CGTGCGGAGT	GAAACCGTGC	GTAAAGAACGT	CATCATCCAG	CTGAACCGCG	TCGACAACGA	2520
	CCTCGCCCGC	CGCGTCGCGC	TAGCTATCCG	CGTCGAACCC	CCATCCCCGG	ACCCAACCTT	2580
	CTACCACAAC	AAGGCAACCG	TCCCATCCGG	CACCTTCGGC	ACGAATCTCC	TGCGGCTCGA	2640
	CGGGCTGAAA	ATCGCCCTCC	TGACAAGAGA	CGACGGTAGC	TTACAGATCG	CGGAGCAGCT	2700
	CCGGGCGCGC	TTTAAACGCG	CCAACAACAA	AGTAGATATC	GTCCTAGTGG	GCTCATCGCT	2760
45	TGATCCCCAA	CGCGGCGTGA	ACATGACCTA	TTCCGGCGCC	GACGGCTCGA	TCTTCGATGC	2820
	CGTGATCGTC	GTCGGCGGCC	TGCTCACGAG	CGCCTCAACG	CAATACCCAA	GAGGTGCGCC	2880
	GCTCAGGATT	ATTACGGATG	CATACGCGTA	TGGAAAGCCC	GTTGGCGCCG	TCGGTGACGG	2940
	TAGCAATGAA	GCCCTTCGTG	ACGTCCTTAT	GGCCGCTGGT	GGGGATGCGT	CGAATGGGCT	3000
	GGACCAAGCC	GGTGTGTATA	TTTCCAACGA	TGTGAGTGAG	GCCTACGTTA	GAAGTGCTTT	3060
50	GGACGGATTG	ACGGCATATC	GGTTCCTGAA	TCGGTTCCTG	TTGGATAGAA	GCTTGGTATG	3120
	AGGTTTGGGG	CGCAAATATG	GGTTTACTAC	CCCCCCCCC	CCCTTTTTTT	TTTTCTTTT	3180
	CTGTTTTTCC	ATCTTTGTTT	GAGGTAATAT	TGCAGATATC	AGTAAATTGC	GTTTACGAAA	3240
	GCCGGTGTCA	AGCTTCANGA	GGCCTAATTA	ATTGGAAGAG	GAGGTTGAAG	TGAAATCTTG	3300
	GTGTAACAT	AATAATTAT	AATAACTAAT	AACCTATAAT	TAATGTCTAT	TGTAATTTCC	3360
55	TCTCACATTC	AATCTATATT	TGATCCTTGT	CCTTTGTAGC	TGTTTAAATA	TAAGCCAAGA	3420
	GAGACAAATA	ATGATAGATT	AACAAATAT	TGCACACCCA	ATAGGCCTTC	CCTCAGGATA	3480
	TCAGATATTA	TCTATCATGT	TGTAATGATA	CCTCAAAAAT	GCCACAAGCT	TGCCTGATAT	3540
	TGAATATTTA	TATGCTGTAA	ATGTAGGGAA	GAGCGTACCA	TCCAAATAAC	CAGAAAAACA	3600
	TGTTTTAGCT	TAAAACTCA	CTAAGGTCGG	TCGTGTCTAT	TTGAAATGGC	TGCGGCAAGC	3660
	TGACTATCTG	ATAAAAATGT	CTGTATTTC	GCTTCACGAC	GCAATGTTATG	ACTTTCGAAT	3720
60	ATAGATAAAA	CCTGAACGAT	TTAGCCCTGT	TTGGGGGAAA	TAGGGGTTAG	GGGGCGGAGC	3780
	TACATATCAT	TCCCATATGA	CCAAAACTA	AAATAGATAT	ATATATATAT	ATATATATAT	3840
	ACAACACCTT	CAAAAAGGAT	CC				3862

## (2) INFORMATION FOR SEQ ID NO:2:

- 65 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 707 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5 Met Pro Ser Gly Gln Lys Gly Pro Leu Asp Arg Arg His Asp Thr Leu  
 1 5 10 15  
 Ser Asp Pro Thr Asp Gln Phe Leu Ser Lys Phe Tyr Ile Asp Asp Glu  
 20 25 30  
 Gln Ser Val Leu Thr Thr Asp Val Gly Gly Pro Ile Glu Asp Gln His  
 35 40 45  
 Ser Leu Lys Ala Gly Asn Arg Gly Pro Thr Leu Leu Glu Asp Phe Ile  
 50 55 60  
 10 Phe Arg Gln Lys Ile Gln His Phe Asp His Glu Arg Val Pro Glu Arg  
 65 70 75 80  
 Ala Val His Ala Arg Gly Ala Gly Ala His Gly Val Phe Thr Ser Tyr  
 85 90 95  
 15 Asn Asn Trp Ser Asn Ile Thr Ala Ala Ser Phe Leu Asn Ala Ala Gly  
 100 105 110  
 Lys Gln Thr Pro Val Phe Val Arg Phe Ser Thr Val Ala Gly Ser Arg  
 115 120 125  
 Gly Ser Val Asp Ser Ala Arg Asp Ile His Gly Phe Ala Thr Arg Leu  
 130 135 140  
 20 Tyr Thr Asp Glu Gly Asn Phe Asp Ile Val Gly Asn Asn Val Pro Val  
 145 150 155 160  
 Phe Phe Ile Gln Asp Ala Ile Gln Phe Pro Asp Leu Ile His Ala Val  
 165 170 175  
 25 Lys Pro Gln Pro Asp Ser Glu Ile Pro Gln Ala Ala Thr Ala His Asp  
 180 185 190  
 Thr Ala Trp Asp Phe Leu Ser Gln Gln Pro Ser Ser Leu His Ala Leu  
 195 200 205  
 Phe Trp Ala Met Ser Gly His Gly Ile Pro Arg Ser Met Arg His Val  
 210 215 220  
 30 Asp Gly Trp Gly Val His Thr Phe Arg Leu Val Thr Asp Glu Gly Asn  
 225 230 235 240  
 Ser Thr Leu Val Lys Phe Arg Trp Lys Thr Leu Gln Gly Arg Ala Gly  
 245 250 255  
 35 Leu Val Trp Glu Glu Ala Gln Ala Leu Gly Gly Lys Asn Pro Asp Phe  
 260 265 270  
 His Arg Gln Asp Leu Trp Asp Ala Ile Glu Ser Gly Arg Tyr Pro Glu  
 275 280 285  
 Trp Glu Leu Gly Phe Gln Leu Val Asn Glu Ala Asp Gln Ser Lys Phe  
 290 295 300  
 40 Asp Phe Asp Leu Leu Asp Pro Thr Lys Ile Ile Pro Glu Glu Leu Val  
 305 310 315 320  
 Pro Phe Thr Pro Ile Gly Lys Met Val Leu Asn Arg Asn Pro Lys Ser  
 325 330 335  
 45 Tyr Phe Ala Glu Thr Glu Gln Ile Met Phe Gln Pro Gly His Val Val  
 340 345 350  
 Arg Gly Ile Asp Phe Thr Asp Asp Pro Leu Leu Gln Gly Arg Leu Tyr  
 355 360 365  
 Ser Tyr Leu Asp Thr Gln Leu Asn Arg His Gly Gly Pro Asn Phe Glu  
 370 375 380  
 50 Gln Leu Pro Ile Asn Arg Pro Arg Ile Pro Phe His Asn Asn Asn Arg  
 385 390 395 400  
 Asp Gly Ala Gly Gln Met Phe Ile Pro Leu Asn Thr Ala Ala Tyr Thr  
 405 410 415  
 55 Pro Asn Ser Met Ser Asn Gly Phe Pro Gln Gln Ala Asn Arg Thr His  
 420 425 430  
 Asn Arg Gly Phe Phe Thr Ala Pro Gly Arg Met Val Asn Gly Pro Leu  
 435 440 445  
 Val Arg Glu Leu Ser Pro Ser Phe Asn Asp Val Trp Ser Gln Pro Arg  
 450 455 460  
 60 Leu Phe Tyr Asn Ser Leu Phe Glu Lys Gln Phe Leu Val Asn  
 465 470 475 480  
 Ala Met Arg Phe Glu Asn Ser His Val Arg Ser Glu Thr Val Arg Lys  
 485 490 495  
 65 Asn Val Ile Ile Gln Leu Asn Arg Val Asp Asn Asp Leu Ala Arg Arg  
 500 505 510  
 Val Ala Leu Ala Ile Gly Val Glu Pro Pro Ser Pro Asp Pro Thr Phe  
 515 520 525  
 Tyr His Asn Lys Ala Thr Val Pro Ile Gly Thr Phe Gly Thr Asn Leu  
 530 535 540  
 70 Leu Arg Leu Asp Gly Leu Lys Ile Ala Leu Leu Thr Arg Asp Asp Gly

[illegible]

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30 Ser Asp Pro Thr Asp Gln Phe Leu  
1 5

(2) INFORMATION FOR SEO ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

40    Asp Phe Ile Phe Arg Gln Lys Ile Gln His Phe Asp His Glu Arg  
          1                  5                  10                  15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Thr Leu Gln Gly Arg Ala - Leu Val  
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear



(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Gln Ala Leu Gly Gly Lys Asn Pro Asp Phe His Arg Gln Asp Leu  
1 5 10 15

5 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Gly Arg Tyr Pro Glu  
1 5

15 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe Asp Phe Asp Leu Leu Asp Pro Thr Lys  
1 5 10

25 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ile Ile Pro Glu Glu Leu Val Pro Phe Thr Pro Ile Gly Lys  
1 5 10

35 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: Other

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AARAAYCCVG AYTYY

15

45 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: Other

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTNCCDATNG TRAA

14

(2) INFORMATION FOR SEQ ID NO:12:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGGAATCCTC CGACCCTACG GA

22

(2) INFORMATION FOR SEQ ID NO:13:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCAAGCTTC TATCCAACGG GAACCGA

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